VOLUME 101 • FEBRUARY 1958 • NUMBER 2

A.M.A. ARCHIVES OF
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WILLIAM B. CASTLE NUMBER

This issue is dedicated to Dr. William B. Castle in honor of his sixtieth birthday. It is made up of contributions from his many colleagues and students.

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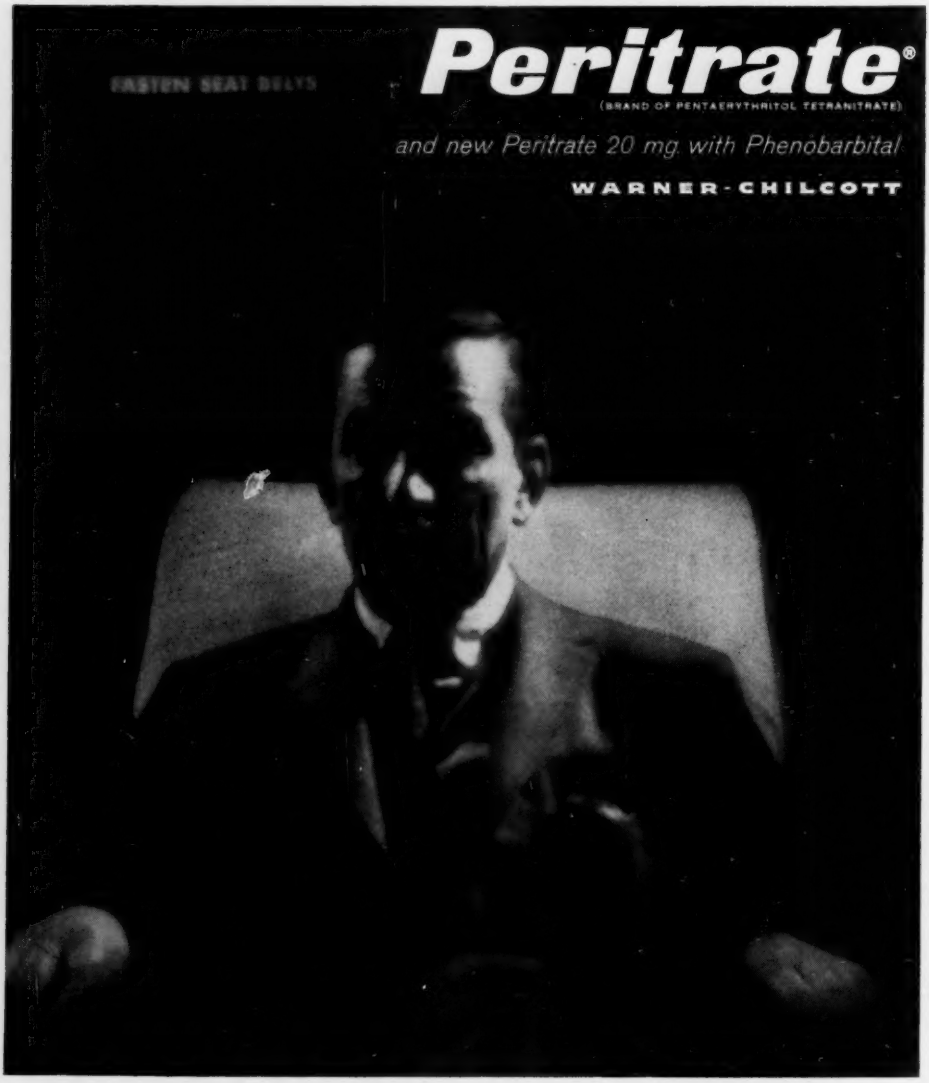

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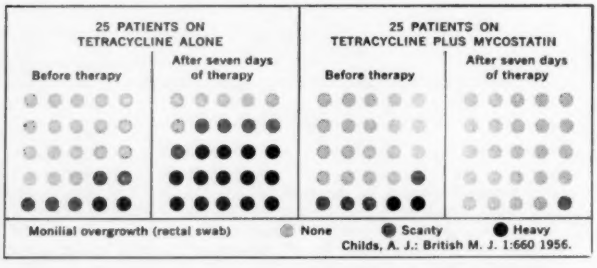

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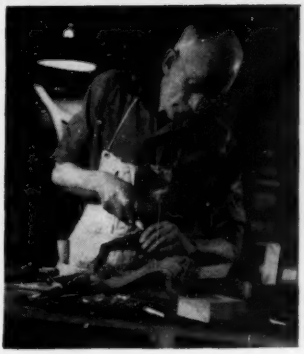

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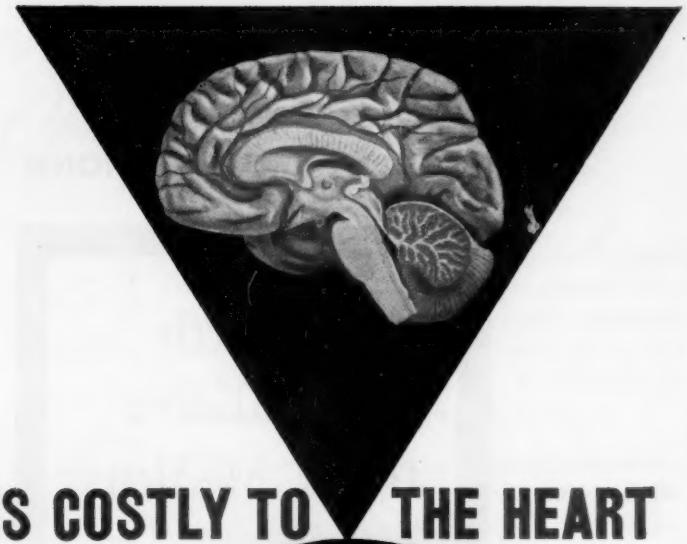

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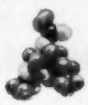

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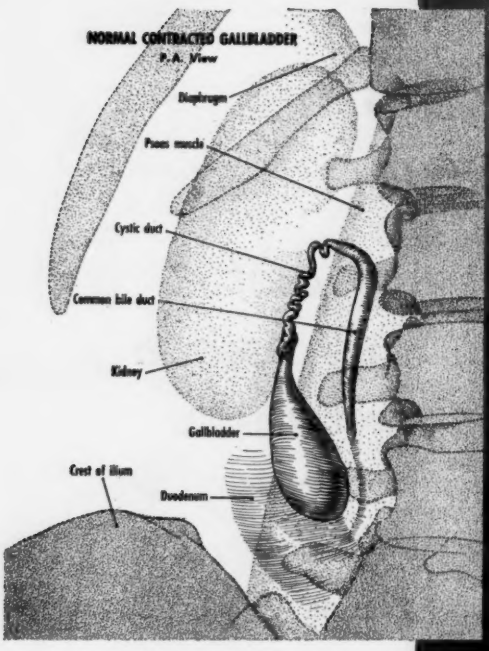

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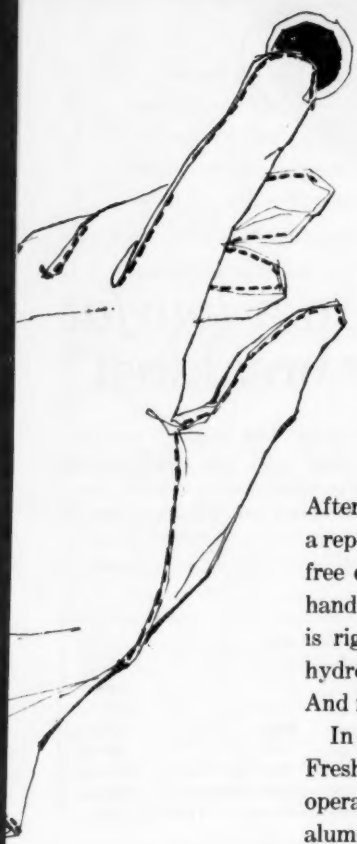

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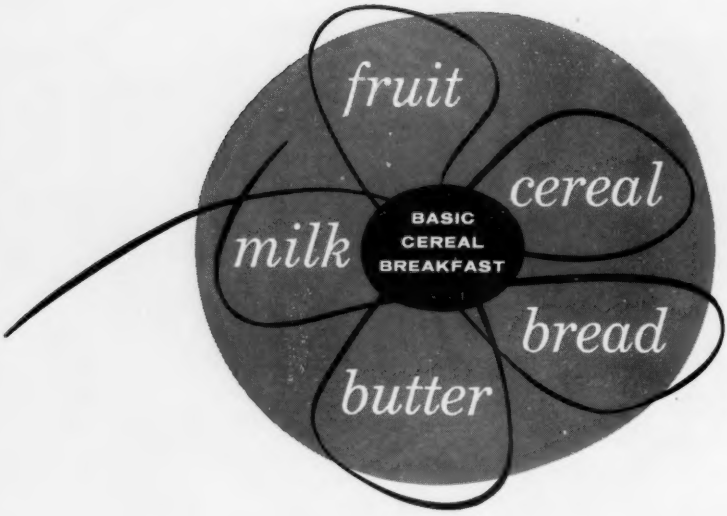

are you interested in a *low-fat* quick and lasting energy breakfast?

When a reduction of fat in the diet is indicated in the morning meal, the fat calories can be replaced by those of *low-fat content* yet providing well-balanced nourishment and quick and lasting energy throughout the morning hours.

The Iowa Breakfast Blood Sugar Studies proved that a basic cereal and milk breakfast providing about 20 gm. mixed plant and animal protein (cereal and milk) provided quick and lasting energy throughout the early and late morning hours. As shown in the table below this morning meal is *low in fat* and provides well-balanced nourishment.

basic cereal low-fat breakfast pattern

Orange juice, fresh, ½ cup,
Cereal, dry weight, 1 oz.,
with whole milk, ½ cup, and sugar, 1 tsp.,
Bread, white, 2 slices, with butter, 1 tsp.,
Milk, nonfat (skim), 1 cup,
black coffee

Nutritive value of basic cereal breakfast pattern

CALORIES	502	VITAMIN A	600 I.U.
PROTEIN	20.5 gm.	THIAMINE	0.46 mg.
FAT	11.6 gm.	RIBOFLAVIN	0.80 mg.
CARBOHYDRATE	80.7 gm.	NIACIN	3.0 mg.
CALCIUM	0.532 gm.	ASCORBIC ACID	65.5 mg.
IRON	2.7 mg.	CHOLESTEROL	32.9 mg.

Note: To further reduce fat and cholesterol use skim milk on cereal which reduces Fat Total to 7.0 gm. and Cholesterol Total to 16.8 mg. Preserves or honey as spread further reduces Fat and Cholesterol.

Bowes, A. deP., and Church, C. F.: Food Values of Portions Commonly Used. 8th ed. Philadelphia: A. deP. Bowes, 1956.
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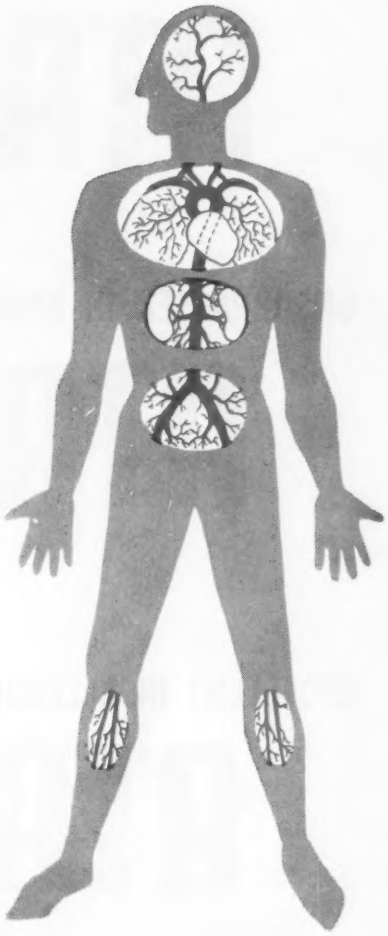

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...LIPO-HEPIN[1] facilitates administration, lowers patient cost, *and* requires only one or two daily injections to establish[2] the desired anticoagulant effect (regardless of patient weight).

[1]Sodium heparin U.S.P aqueous, 2 cc or 10 cc multiple dose vial, 20,000 U.S.P. units (200 mgs.) per cc. For intravenous, intramuscular or subcutaneous use.

[2]Clotting times are not suggested from the standpoint of avoiding danger in either the hospitalized or ambulatory patient when Lipo-Hepin dosage schedule and injection technique is used. Clotting times may be taken during initial therapy to insure adequate effect. (Literature available on request.)

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Dr. William B. Castle, Minot Professor of Medicine, Harvard University.

A.M.A. ARCHIVES OF
INTERNAL MEDICINE

The Editors of the A. M. A. ARCHIVES OF INTERNAL MEDICINE *take great satisfaction in dedicating this issue to William B. Castle in honor of his sixtieth birthday. The papers which follow were submitted by his many colleagues and students of the Harvard Medical Unit of the Boston City Hospital. We join his friends and admirers in wishing him well on this anniversary. In addition to our admiration for his own brilliant achievements we congratulate him on having had a hand in the training of an outstanding group of men, some of whose contributions follow.*

P. S. R.

Foreword

Important milestones such as Dr. Castle's sixtieth birthday provide a pleasant release from customary reticence, enabling one to express more freely the affection and admiration for the birthday celebrant. Actually, 1957 marks more than one anniversary in Dr. Castle's life. It is the fortieth anniversary of his matriculation as a student in the Harvard Medical School, the twentieth anniversary of his appointment as Professor of Medicine at Harvard University, and the tenth anniversary of his appointment as Director of the Thorndike Memorial Laboratory while serving as Director of the Second and Fourth (Harvard) Medical Services of the Boston City Hospital. And now, a further event makes this year memorable, namely, his appointment as the first George Richards Minot Professor of Medicine.

During the decades of Dr. Castle's leadership of his department of medicine, luster has been added to the already rich tradition of Francis W. Peabody and George Richards Minot. The program of undergraduate and postgraduate education has flourished, and the Thorndike Memorial Laboratory has continued to be a center for distinguished research. From 1922 until the present time, more than four hundred young physicians have received postgraduate training under Dr. Peabody, Dr. Minot, and Dr. Castle; many of them have subsequently achieved important positions in medical education and in research in the United States and abroad. Approximately one hundred twenty of these graduates are now serving as Deans, Professors, or Associate Professors, and numerous others are actively participating in academic life.

In these men, as indeed in all of us who have known him, Dr. Castle's rare qualities of mind and heart, evident from the days he and I were medical school classmates, have evoked admiration and inspiration. These qualities were epitomized in the citation on the occasion when Dr. Castle was awarded the honorary degree of Doctor of Science by the University of Chicago: "... his inspired clinical studies have made fundamental contributions to our knowledge of blood formation. His studies on pernicious anemia which began more than twenty-five years ago and culminated in an understanding of the defect responsible for this disease stand out as a classic in clinical investigation. His important findings about the factors influencing blood formation have been the basis for a continu-

ing growth of knowledge in this field. He is possessed of several attributes rarely found in a single individual—a scholarly approach to clinical investigation, a devotion to the opportunity of teaching the beginner as well as the graduate, and a great skill as a practitioner of the healing arts."

To these qualities of a distinguished scientist, an astute clinician, an inspiring teacher, and a warm humanitarian may be added humility, wisdom, and humor.

The tributes of affection that have poured forth from this country and from foreign lands testify eloquently to the rejoicing in Dr. Castle's attainment of threescore years and their fervent wishes that many tens will follow. For many hundreds of his pupils and associates, an ancient observation is verified: "A faithful friend is a strong defense; and he that hath found one, hath found a treasure."

HERRMAN L. BLUMGART, M.D.

William Bosworth Castle

Curriculum Vitae and Bibliography

Born:	Oct. 21, 1897, Cambridge, Mass.
1909-1914	Browne and Nichols School, Cambridge, Mass.
1914-1917	Harvard College, no degree
1921	M.D. Harvard University
1933	S.M. (Hon.) Yale University
1936	M.D. (Hon.) University of Utrecht
1952	S.D. (Hon.) University of Chicago
1921-1923	Medical Intern, Massachusetts General Hospital
1923-1924	Assistant in Physiology, Harvard School of Public Health
1924-1925	Instructor in Physiology, Harvard Medical School, and Assistant in Physiology, Harvard School of Public Health
1925-1926	Assistant Resident, Thorndike Memorial Laboratory, Boston City Hospital
1925-1927	Assistant in Medicine, Harvard Medical School
1926-1927	Chief Resident, Thorndike Memorial Laboratory, Boston City Hospital
1927-1929	Assistant in Medicine, Thorndike Memorial Laboratory, Boston City Hospital
1927-1928	Alumni Assistant in Medicine, Harvard Medical School
1928-1929	Assistant in Medicine, Harvard Medical School
1929	Instructor in Medicine, Harvard Medical School (September to December, 1929)
1929-1932	Assistant Professor of Medicine, Harvard Medical School
1929-1948	Associate Physician, Thorndike Memorial Laboratory, Boston City Hospital
1930-1932	Assistant in Medicine, Boston City Hospital
1931-1932	Director, Rockefeller Foundation Commission for Study of Anemia in Porto Rico
1932-1933	Assistant Physician, Boston City Hospital
1932-1937	Associate Professor of Medicine, Harvard Medical School
1932-1948	Associate Director, Thorndike Memorial Laboratory, Boston City Hospital
1933-1948	Junior Visiting Physician, Boston City Hospital
1936-1948	Chairman of the Department of Medicine, Harvard Medical School
1937-1957	Professor of Medicine, Harvard University
1940	Director, Second and Fourth (Harvard) Medical Services, Boston City Hospital
1947	Member, Consulting Staff, Faulkner Hospital
1947	Consultant in Medicine, Beverly Hospital
1948	Director, Thorndike Memorial Laboratory, Boston City Hospital (July 1)
1948-1955	Assistant Visiting Physician, Boston City Hospital (Nov. 17 to Dec. 31)
1950	Consultant in Research, Boston State Hospital
1955	Senior Consultant in Hematology, Lemuel Shattuck Hospital
1956	Visiting Physician, Boston City Hospital (Jan. 1)
1956	Consultant in Medicine, Beth Israel Hospital
1957	George Richards Minot Professor of Medicine, Harvard University

Medical Societies

Fellow of The American Academy of Arts and Sciences
The American College of Physicians (Master, since April, 1957)
Member of The American Academy of Tropical Medicine (dissolution of Academy November, 1954)
The American Association for the Advancement of Science
The American Medical Association
The American Philosophical Society
The American Society for Clinical Investigation (President 1940-1941) (Emeritus since 1943)
The American Society for Experimental Pathology
The American Society of Tropical Medicine and Hygiene
The Association of American Physicians

The Boston Society of Biologists
The Massachusetts Medical Society
The National Academy of Sciences
The Society for Experimental Biology and Medicine
Senior Member, American Federation for Clinical Research
Correspondant étranger, l'Académie royale de Médecine de Belgique
Correspondant membre, Société Internationale Européenne d'Hématologie
Honorary Member, Section of Medicine of The Royal Society of Medicine
Honorary Fellow, Royal Australasian College of Physicians (1954)
Foreign Honorary Member, Societas Medicorum Finlandiae (November, 1954)
Foreign Corresponding Member, British Medical Association (April, 1957)

Honorary Societies

Phi Beta Kappa (1918)
Alpha Omega Alpha (1920)

Special Awards

The Warren Triennial Prize, Massachusetts General Hospital, 1928
The John Phillips Memorial Prize of the American College of Physicians for the year 1932
The William Procter Jr. International Award for Distinguished Service in the Sciences Promoting Health, by the Philadelphia College of Pharmacy and Science, for the year 1935
The Walter Reed Medal of the American Society of Tropical Medicine, in recognition of meritorious achievement in the field of tropical medicine, Nov. 21, 1939
The Major General Leonard Wood Medal, by the Boston City Hospital Alumni Association, in recognition of contribution to medicine and humanity through work in pernicious anemia, April 30, 1949
The Mead Johnson and Company Award for Research on the Vitamin B Complex, in recognition of classic investigations leading to the concept of intrinsic and extrinsic factors in pernicious anemia, and of studies elucidating the relation of vitamin B_{12} to this concept, April 19, 1950

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The Shock Syndrome Associated with Bacteremia Due to Gram-Negative Bacilli

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Antibiotics have caused remarkable changes in the incidence and management of infectious diseases. Infections due to Gram-positive organisms, notably those caused by pneumococci and streptococci, have diminished in incidence and in severity. On the other hand, this decline has been paralleled by a decided increase both in the frequency and in the severity of disease caused by Gram-negative bacteria, including the coliform group.[1-9]

This changing pattern has been attributed to at least two factors. First, the Gram-positive bacteria are effectively suppressed by chemotherapeutic agents. Secondly, there has been the emergence of an increasingly larger proportion of Gram-negative micro-organisms that are highly resistant to the action of available antibiotics.[1-4] Studies in individual patients have shown that the relatively nonpathogenic strains of bacteria, such as Escherichia coli, Proteus vulgaris, Aerobacter aerogenes, or Pseudomonas aeruginosa, may actually invade and cause infection during the course of antibiotic treatment.[5-9] This paradox has been attributed to the elimination of competitive Gram-positive organisms by the antibiotics and then the uninhibited multiplication of the symbiotic Gram-negative bacilli. Elderly patients being treated with antibiotics for unrelated acute infections appear to be particularly susceptible to superinfection by the coliform group of organisms. Similarly, patients convalescing from surgical procedures or who have severe debilitating diseases also seem to be more frequently infected by these micro-organisms.[2] Following multiplication of these bacteria in infected tissues, invasion of the blood stream commonly occurs. In this manner a relatively benign infection is converted into a more serious infection fraught with hazardous complications.

In 1951, Waisbren [10] reported from this clinic observations on 29 patients with bacteremia due to Gram-negative bacilli. He recognized two patterns of illness in these patients. In one group, of 14 patients, signs of sepsis predominated. These persons appeared hot, dry, flushed, and alert. The other group, of 15 patients, were described as cold, clammy, apprehensive, and lethargic. Hypotension and the other signs of shock predominated. Of the 29 patients reported, 5 died, and 4 of these had the picture of shock.

Bacteremia has become the commonest single cause of shock, other than shock due to myocardial infarction, now being treated on the medical services at the University Hospitals. The increasing clinical significance of this condition has led to an extensive study of the underlying mechanisms involved. The present report represents a clinical inquiry and was designed to complement the experimental investigations [11-12] on the cause and nature of this type of shock. It is based on a review of observations on 400 patients who had blood cultures from which Gram-negative micro-organisms were grown. Shock occurred in 43 of the patients, and these cases were studied in detail. In considering this material, special attention was focused on the events that preceded and may have contributed to the

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development of bacteremia and shock: the early diagnostic features, the clinical course, and the results of therapy.

Methods of Study

The 400 patients were observed at the University of Minnesota Hospitals between Jan. 1, 1950, and Sept. 30, 1955, and included all patients who had one or more blood cultures from which Gram-negative micro-organisms were grown in broth alone or in broth and on nutrient agar. The cultures were identified by the diagnostic bacteriology laboratory of the University Hospitals. In 278 of the 400 cases, the bacteriological findings correlated with the clinical course, indicating that bacteremia existed and that the recovery of organisms was not the result of contamination.. In 43 patients with proved bacteremia, hypotension and other signs of shock occurred. This was reflected by an average fall in arterial pressure from an admission value of 134/78 to 63/26 mm. of Hg as measured by the clinical sphygmomanometer. In each instance, the blood pressure fell to below 90 mm. systolic, except in the case of one patient with severe hypertensive vascular disease, who displayed the clinical features of shock following a decline in systolic blood pressure from 220 to 146 mm. of Hg. For present purposes, "total group" refers to the initial group of 400 cases having positive blood cultures. "Bacteremia" designates 278 of these 400 patients in whom the clinical features left no reasonable doubt that a systemic infection with invasion of the blood stream actually existed. The 43 cases in which bacteremia was complicated by hypotension are referred to as patients with "shock."

TABLE 1.—*Patients Classified According to Hospital Services, Including Those Whose Blood Cultures Yielded Organisms, Those with Established Infections and Bacteremia, and Those with Bacteremic Shock*

Hospital Service	Patients with Positive Blood Cultures		Patients with Established Infections and Bacteremia		Patients with Bacteremic Shock	
	No.	%	No.	%	No.	%
Medicine	106	27	76	27	15	35
Surgery	100	25	78	28	18	42
Urology	89	22	61	22	8	19
Pediatrics	63	16	36	13	0	0
Neurology	21	5	14	5	1	2
Other	21	5	13	5	1	2
Totals	400	100	278	100	43	100
Percentage of total (400 patients)		100		69		11

Results

Age.—Although 36 cases of bacteremia (13%) occurred in the pediatric group, including the ages up through 15 years, no instance of shock was observed in these patients (Table 1). The youngest patient with shock was 34 years old. The median age of the total group was 58 years; for patients with bacteremia, 64 years, and for patients with shock, 65 years.

Sex.—Shock occurred in 28 male and 15 female patients. This sex difference may be ascribed to the disproportionate incidence of urinary tract disorders occurring in the elderly man and the importance of urethral instrumentation as a precipitating cause.

Incidence Classified According to Hospital Services.—The incidence of bacteremic shock was highest on the surgical service. The condition occurred most frequently in patients who were recuperating from extensive abdominal surgery. The incidence on the medical service was almost equal to that on surgery (Table 1). The medical patients had associated serious and debilitating systemic disease, such as carcinomatosis, agranulocytosis, or uncontrolled diabetic acidosis. Considering that there were less than half as many total patients on the urologic service, as contrasted to the surgical or medical services, the incidence of bacteremia was approximately twice as high. On the other hand, urological patients with bacteremia manifested shock only about one-half as frequently.

TABLE 2.—*Presumed Precipitating Incidents in Forty-Three Cases of Bacteremic Shock* *

	No.	%
Manipulations of urinary tract	20	43
Abdominal surgery	10	22
Intravenous infusion or cannulation of peripheral vein ("cut-down")	6	13
Dressing or other manipulation of infection involving skin or subcutaneous tissues	3	6
Perineal and pelvic surgery	3	6
Paracentesis	1	2
Administration of antibiotic during bacteremia	2	4
Unknown	2	4
Total	47	100

* In four patients two events were temporally related to the onset of shock.

Precipitating Factors.—Only 3 of the 43 instances of shock occurred before the patients were admitted to the hospital. One patient was admitted from a nursing home, where he had become markedly hypotensive shortly after an intravenous infusion of dextrose solution, which presumably was contaminated. The other two patients had chronic urinary tract infections requiring indwelling urethral catheters.

In almost every case, the development of bacteremic shock was related to a procedure conducive to trauma (Table 2). Frequently, this involved relatively simple procedures, such as insertion of a urethral catheter, cannulation of a peripheral vein to supplement fluid therapy in seriously ill patients, administration of intravenous fluids under unfavorable conditions with respect to aseptic technique, dressing of wounds, and following a paracentesis. Instrumentation of the urinary tract was implicated as the most frequent cause and occurred in 20 instances (43%). In 14, or 70%, of these patients, the same species of bacteria was demonstrated in the blood stream and in the urine. The onset of shock in two patients occurred shortly after initiation of antibiotic therapy. This was reminiscent of that which was observed in critically ill patients with brucellosis shortly after they had been given the first dose of chlortetracycline.[13] The untoward reactions were presumed due to the liberation of endotoxin from the disintegrating brucellae.

The average interval between the time of admission to the hospital and the occurrence of shock was 17 days,* indicating the likelihood that events in the hospital were responsible. An average period of 25 hours intervened between the causative incident and the onset of an abrupt rise in temperature. Approximately 13 additional hours elapsed before shock was manifested. In 23% of the patients shock and fever occurred simultaneously, and in 12% no significant change in temperature was associated with the events that terminated in bacteremic shock.

TABLE 3.—*Clinical Features Noted in Forty-Three Cases of Shock Caused by Gram-Negative Bacteremia*

	%
Hypotention	100
Increase in polymorphonuclear leukocytes	86
Increase of total leukocyte count	83
Elevation of blood urea nitrogen	81
Temperature above 101 F (38.3 C)	77
Electrocardiographic abnormalities	74
Impaired mental state	67
Chills	58
Diarrhea	54
Vomiting	54
Abnormal serum electrolytes	49
Urine output below 500 cc./24 hr.	33
Abdominal cramps	14
Warm perspiration	14
Jaundice	7

Clinical Features.—Shock was typically, but not invariably, preceded by chills, a spiking febrile reaction, and profuse perspiration (Table 3). The onset of hypotension was frequently recognized by the nursing staff because of the sudden onset of mental confusion. A rapid thready pulse, pallor, cold and moist skin, and collapsed superficial veins were noted in most cases, but six patients (14%) had warm, dry, or moist skin during shock. Vomiting and the expulsion of large stools which were usually mucoid or watery accompanied the onset of shock in most patients. Bloody vomitus was observed in seven cases (18%), after exclusion of five cases in which hematemesis could conceivably have presented as a complication of surgery of the gastrointestinal tract. Bloody diarrhea also occurred in seven patients, excluding seven additional cases in which melena may have been related to surgery. In six patients (14%), severe abdominal cramps were associated with the onset of shock.

The leukocyte count, 6 to 48 hours after onset of shock, was elevated in all but five cases, and the number of polymorphonuclear leukocytes was increased in all except three cases. The average value following the onset of shock was 13,305 white cells per cubic millimeter, and the average increase in neutrophil percentage was 20%.

* The average hospitalization period at the University Hospitals for all patients during the same period was 13 to 14 days.

TABLE 4.—*Mortality Rates for All Patients*

Classification of Patients	No. Total Cases	No. Died	Mortality, %
Those with positive blood culture	122	43	35
Those with established infection and bacteremia	235	94	40
Those with bacteremic shock	43	28	65
Total	400	165	41

A frequent accompaniment of shock was the rapid appearance of uremia and acidosis. Significant electrolyte disturbances occurred in approximately one-half of the patients. The commonest pattern revealed a loss of fixed base, which resulted in metabolic acidosis. Cheyne-Stokes respiration and hyperventilation further complicated the electrolyte picture as the result of varying degrees of respiratory acidosis and alkalosis. The blood urea nitrogen exceeded the normal limit of 25 mg. per 100 ml. in 81% of the patients with shock, 50 mg. in 54%, and 100 mg. in 19%. The output of urine decreased to less than 500 ml. per day in 33% of patients with shock. Death was directly attributable to uremia in four cases (9%), but no patients died during the oliguric phase.

Twenty-five of thirty-four patients (74%) had significant electrocardiographic abnormalities, the extent of the changes corresponding to the duration of hypotension. The most frequent alterations involved ST- and T-wave changes, suggesting myocardial ischemia and injury. Atrioventricular conduction disturbances also were commonly seen, and these were frequently related to the use of sympathicomimetic drugs. In a majority of these patients, a diagnosis of myocardial infarction was entertained before bacteremia was implicated. Only one patient had congestive heart failure during the phase of shock.

TABLE 5.—*The Incidence and Mortality of Bacteremic Shock According to the Species of Bacteria That Caused the Condition*

	All Positive Blood Cultures				Bacteremia with Shock			
	Total		Lethal		Total		Lethal	
	No.	% *	No.	% †	No.	% *	No.	% †
Pseudomonas aeruginosa	125	28	44	35	8	17	3	38
Proteus sp.	97	22	41	42	15	32	7	47
Escherichia coli	96	21	37	26	7	15	5	71
Aerobacter aerogenes	59	13	35	59	8	17	5	63
Paracolobactrum sp.	35	8	18	51.5	8	17	6	75
Alkaligenes sp.	31	7	9	29	0	0	0	0
Salmonella sp.	4	1	0	0	1	2	0	0
Total	400	100	165	--	47	100	26	--

* Referred to the total group of 400 patients.
† Referred to the number of infections caused by that organism.

Jaundice, not otherwise explained, developed insidiously with shock in three patients (7%). No petechiae, echymoses, or other hemorrhagic phenomena, except for gastrointestinal bleeding, were observed.

Mortality and Prognosis.—There was a progressive increase in death rate when the groups with positive blood cultures, those with established infections and bacteremia, and those with shock were contrasted (Table 4). Among the 43 patients with shock, 28 (65%) died, and 23 (54%) died in shock in an average of 2.7 days (7 hours to 10 days) after the onset of hypotension. Five patients (12%) survived the acute episode of shock but died subsequently. The latter were included as fatal cases of shock for purposes of calculation. Although there is not doubt that bacteremia and shock in these five patients contributed to the fatal outcome, the principal cause of death was more directly related to the primary disease for which these patients were hospitalized. The mean duration of the state of shock in patients who recovered was 6 days (12 hours to 26 days), which indicates the protracted course of this type of shock.

Bacteriologic Findings.†—Of the total group of 400 patients, 84% of the bacteria isolated were classified as Pseudomonas, Proteus, Escherichia, and Aerobacter, in that order. In cases of bacteremic shock, the Proteus species were isolated twice as frequently as any other, but the highest mortality rate was associated with Paracolobactrum organisms. These findings are summarized in Table 5.

† These data were made available by Drs. Robert Wise and Robert Abernathy, formerly in charge of the Diagnostic Bacteriology Laboratory in the University of Minnesota Hospitals.

Two species of organisms were cultured in the blood of 47 of the 400 cases with positive blood cultures. It was of interest that the incidence of bacteremic shock among these 47 cases was 24%, in contrast to 11% for the total group of 400. Moreover, the mortality rate increased from 65% to 82% in patients in shock and infected with two species of organisms.

The mortality rate in eight cases where a maximum of five colonies were grown from 1 ml. of blood was 50%. In the 19 instances where more than five colonies of coliform bacteria appeared on the poured agar plate, the mortality was 84%.

Therapy.—Antibiotics were selected and administered in most cases before precise bacteriological data became available. In vitro sensitivity tests, performed by the tube-dilution method, were done in 12 cases. Three of five patients who had received effective therapy, as determined by subsequent in vitro tests, recovered. On the other hand, no patient in the group of seven who received inappropriate antibiotics, as adjudged by these tests, recovered.

No statistical analysis of the value of vasopressor amines was possible because the decision to use these drugs and the specific agent selected were highly selective. Sympathicomimetic agents, such as epinephrine, ephedrine, phenylephrine (Neo-Synephrine) and methoxamine (Vasoxyl), were employed before levarterenol (Levophed) became available for general use, in 1953. However, these agents were usually ineffective for prolonged treatment of patients with profound shock. More recently, levarterenol and metaraminol (Aramine) have been used. They were employed for treatment of patients who were more severely ill, and this is reflected in the mortality data presented in Table 6.

Corticotropin (ACTH), cortisone, hydrocortisone, or adrenal extract was given to 17 critically ill patients. In several instances, striking improvement occurred soon after the intravenous infusion of hydrocortisone. In two instances, it was possible to demonstrate that the patient's response to the vasopressor agents was simultaneously improved. Chills, fever, vomiting, diarrhea, and other signs of toxemia were decreased. The data presented in Table 6 are not definitive and only suggest that the use of corticotropin and the adrenal steroids was associated with a decrease in the mortality rate from bacteremic shock.

The correction of electrolyte disturbances was occasionally helpful in reestablishing a good response to vasopressor therapy. A refrigeration blanket, as described by Lewis, Ring, and Alden,[14] was used to control high fever and to produce mild hypothermia in three patients. Although only one patient recovered, this therapeutic technique appeared to have a beneficial effect on the course of shock.

Prophylaxis.—There is considerable question as to whether protection was offered by prophylactic therapy with antibiotics. Of the 43 patients who developed shock, a majority had received chemotherapy for 24 or more hours before the precipitating event. Twenty (46%) had received a combination of penicillin, in a dose of 1,200,000 units, and 1 gm.

TABLE 6.—*Therapeutic Results with Vasopressor Agents With and Without Adrenocortical Therapy*

	With Adrenocortical Therapy		Without Adrenocortical Therapy		Total No.	No. Died	% Died
	Survived	Died	Survived	Died			
Levarterenol	2	3	2	8	15	11	73
Metaraminol	2	3	2	3	10	6	60
Other vasopressor agents	2	3	1	0	6	3	50
Levarterenol and metaraminol	1	1	0	3	5	4	80
Total	7	10	5	14	36	24	67
Total	17		19		36		
Per cent died	59		74		67		

of streptomycin per day. Moreover, 25 (58%) had received one or more broad-spectrum antibiotics, most frequently, tetracycline. It was not possible to assess the possible detrimental role played by these drugs in permitting the establishment of infections by antibiotic-resistant species.

Comment

It is generally accepted that shock associated with infection of the blood stream by Gram-negative bacteria is related to the liberation of endotoxin from the bacteria. Characteristics of the circulatory defect that is produced by endotoxins have been studied in laboratory animals, particularly in the dog.[11-12,15-17] Considerable caution is necessary in relating such laboratory studies to the shock syndrome which occurs in human patients. However, the findings in animals have supplied provisional explanations for some of the perplexing features that have been encountered at the bedside.

The reactions of animals caused by endotoxin from various species of Gram-negative organisms are strikingly similar. When purified endotoxins such as those derived from Brucella melitensis, Salmonella typhi, or E. coli are injected intravenously into adult dogs, a characteristic sequence of events follows. As soon as the injection is completed, the animal has a forceful bowel movement. Two or three minutes later, pruritus, hyperpnea, agitation, vomiting, and diarrhea become manifested. During this time the animal shivers markedly and the blood pressure falls progressively. During the first hour, gagging and tenesmus are prominent, and this is later followed by bloody vomitus and bloody diarrhea. In 3 to 5 hours, the animals become comatose, and death occurs at approximately 18 hours. On autopsy, severe congestion of the liver, submucosal bleeding, and surface epithelial slough of large portions of the small intestine are observed.[15]

The hemodynamic disturbances that accompany these clinical changes have also been studied. Shortly after injection of endotoxin, the arterial pressure falls markedly, and this occurs simultaneously with a notable increase in the portal vein pressure. A drastic reduction of cardiac output to approximately one-third its control value is found, but the peripheral resistance remains essentially unchanged. Measurement of the inflow of venous blood into the right atrium indicates that the venous return is reduced in proportion to the decline in cardiac output. The primary disturbance actually occurs on the venous side, and by measurements of the blood contained in various organs, the splanchnic venous bed and especially the liver are shown to be sites where large quantities of blood are pooled. Thus, shock in the dog results from the removal of a large amount of blood from the active circulation due to pooling in distended venous beds. There is no evidence that shock is related to a loss of arteriolar tone, diffuse injury of the capillary bed, or primary failure of the heart.[12] In addition, this pooling occurs in the decapitated dog preparation, thereby demonstrating that it is not caused by a primary effect on higher nervous centers.[15]

In human patients the sudden onset of shock with a high incidence of gastrointestinal manifestations is similar to the response seen in the experimental animal. In several reported instances of shock in human patients, which have occurred following the accidental administration of blood contaminated with a heavy inoculum of Gram-negative bacteria, it is noteworthy that there was a sudden fall in arterial pressure, hematemesis and melena, and a rapid fatal course.[18-20]

Nineteen of the forty-three patients on whom the present study is based were examined at autopsy. It is of interest that in these human cases congestion of the splanchnic organs and mucosal hemorrhages of the intestine were prominent. Microscopic examination both in human material and in dogs following shock due to endotoxin revealed vascular lesions consisting of intraluminal and subendothelial hyaline fibrinoid

material. These findings suggest that the changes which accompany shock in the dog and those in human patients are not dissimilar.

From the information available at present, it can be postulated that removal of blood and its storage in inactive venous pools is responsible for shock. In many respects shock produced by bacterial products simulates anaphylactic shock.[22]

The clinical features of bacteremic shock are rarely distinctive enough to allow for an unequivocal and prompt diagnosis. Awareness of the existence of a source of infection and a prompt analysis of the clinical events that occurred in the period of 72 hours preceding the onset of hypotension are helpful. In our experience, the temperature chart and the nurses' notes offered informative data. An event such as urethral catheterization or dressing of a wound, not necessarily done by a physician, is usually recorded. Within 24 hours of this notation, a spike in temperature commonly occurs, and this is often accompanied by a chill. Signs of sepsis, including fever, elevation of the leukocyte count with a disproportionate increase in the percentage of neutrophils, the occurrence of anorexia, and the development of malaise, usually precede but sometimes accompany the insidious onset of hypotension. Nausea, vomiting, diarrhea, and/or abdominal pain occur in a majority of the patients, and the onset of the gastrointestinal disturbances usually coincides with the onset of the state of shock. Because of the frequency with which electrocardiographic changes, and especially evidence of myocardial ischemia, are detected in these elderly patients with shock, the differentiation between myocardial infarction and bacteremic shock may be difficult. The absence of chest pain, the presence of gastrointestinal signs, and a consideration of historical features may aid in differential diagnosis. It is of primary importance that a blood culture is obtained early if the diagnosis is suspected, not only to obtain bacteriologic confirmation but also to guide the selection of chemotherapeutic agents.

As in previous studies,[10,23-24] the genitourinary tract was the most frequent source of the bacteremia. In more than 90% of the cases cited in the present report, bacteremia was initiated by an event that occurred in the hospital. From the present analysis, prophylactic antibiotic therapy yielded disappointing results. The majority of patients received antibiotics preceding the event presumed to be responsible for bacteremia and the subsequent development of shock. Not only may antibiotics fail to protect the patient, but several workers have advanced cogent argument that susceptibility may actually be enhanced by infection with hospital-acclimatized organisms that are highly resistant to antibiotics.[1,3,9]

The results of therapy in a group of patients with bacteremic shock are difficult to evaluate because controlled observations are rarely available. The judicious choice and early use of antibiotic drugs is of the greatest importance. It is evident that shock often persists in spite of sterilization of the blood stream. Indeed, antibiotic therapy may itself induce shock during treatment of bacteremia, presumably by the sudden destruction of bacterial cells and liberation of relatively large amounts of endotoxin from the lysed bacteria.[13] This situation is uncommon, however, and the outlook, favorable. But the outlook is grim when massive infection continues to be uncontrolled for a prolonged period in the presence of shock. In instances where more than five colonies of bacteria are grown from 1 ml. of blood and the patient receives an inappropriate antibiotic, as judged on the basis of in vitro sensitivity testing, survival is most unlikely. These findings confirm similar observations previously made by Hall and Gold.[23]

The use of corticosteroid drugs and vasopressor agents was generally reserved for those patients in whom response to antibiotics, fluids, and other supportive treatment proved inadequate. These patients were critically ill, and the prognosis was grave.

Administration of adrenal cortical hormones was usually followed by improvement in the appearance of the patient.

The value of vasopressor agents in the treatment of certain types of shock has been widely recognized. In experiments with dogs, metaraminol has been shown to remedy the venous pooling that follows injection of endotoxin.[16] In human patients with bacteremic shock, amines such as levarterenol [25-27] and metaraminol [27] produce a prompt elevation of the arterial pressure, adjustment of the pulse to a slower rate and fuller volume, and improvement in general appearance. Metaraminol appeared to be a more favorable agent because of its prolonged action, the absence of local tissue injury, and the possibility of subcutaneous and intramuscular administration during the later phases of therapy.[23,27] The need for only very small quantities of fluid vehicle for the administration of metaraminol was a special advantage for patients with poor renal function.

The frequency with which renal complications occur following bacteremic shock merits special mention. Hinshaw and his co-workers [28] studied the effect of endotoxin on the isolated and perfused kidney of the dog and found that the endotoxin produced profound disturbances in renal function. Moreover, in the intact host reduction in renal blood flow as a consequence of arterial hypotension can be expected to produce renal ischemia and damage not unlike that which occurs in post-traumatic shock.[29] Finally, pyelonephritic abscesses, glomerulitis, and renal infarcts are produced by dissemination of the bacteria and occurred in a majority of the cases that were studied at autopsy.[21] The problem of acute renal failure does not usually become a limiting factor to survival until recovery from bacteremia and shock has been achieved. Its management requires careful attention to fluid and electrolyte balance and maintenance of proper caloric intake. If infection has been controlled, dialysis with use of the artificial kidney may be considered, although there was no occasion to use this technique in the present series of cases.

A matter of great interest has been the age of patients who develop bacteremic shock. Not a single instance of this type of shock occurred on the pediatric service. The youngest of 43 patients studied was 34 years, and the youngest of 22 patients who had shock in association with bacteremia due to Gram-negative micro-organisms in the series reported by Hall and Gold [23] was 44 years. In experiments with dogs, puppies were much more resistant to shock produced by endotoxin than adult dogs.[15] Smith and Thomas [30] noted that young rabbits are much more resistant to the lethal action of meningococcal toxin. This difference in susceptibility between the immature animal and the adult remains entirely unexplained, and future classification of these seemingly important differences may well provide an important link for our understanding of the entire problem.

Summary and Conclusions

A study was made of the clinical features of the 400 patients who were hospitalized at the University of Minnesota Hospitals in the period between January, 1950, and September, 1955, and from whose blood Gram-negative enteric bacteria were recovered. In 278 of these cases there was no reasonable doubt that bacteremia existed. Bacteremia was complicated by hypotension and the manifestations of shock in 43 of these patients.

In more than 90% of the cases of shock, a procedure performed in the hospital was implicated. This included instrumentation of the lower urinary passages, septic complications of surgery, cannulation of a peripheral vein, administration of intravenous fluids under unfavorable conditions of sterility, and paracentesis. Signs of sepsis, including chills and fever, followed within 48 hours of the causative event, and shock appeared approximately 12 hours later. Nausea, vomiting, and diarrhea usually accompanied the onset

of shock. The leukocyte count and percentage of neutrophils were notably increased. A majority of patients had significant electrocardiographic abnormalities, which in many instances made differentiation from shock caused by myocardial infarction very difficult. It is important to obtain a blood culture in instances where the cause of shock is not apparent. This is of value not only for the establishment of a specific diagnosis but also for planning optimal antibiotic therapy.

The mortality rate of patients with bacteremia and shock was 65%, with death occurring at an average of 2.7 days after onset of shock. The mean duration of the state of shock for patients who survived was 6 days (12 hours to 26 days), indicating the protracted course of this type of shock. Acute renal failure may become a limiting factor during the recovery period and was the cause of death in four cases of the present series.

The prompt use and judicious choice of antibiotics are most important aspects of therapy. Vasopressor agents, and especially metaraminol, probably promote reversibility of the circulatory disturbance and provide effective elevation of blood pressure. Adrenal cortical hormones suppress the manifestations of toxemia caused by overwhelming infection and may be used to provide a period of grace during which infection and shock can be controlled.

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Loafer's Heart

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R. Price's ironical phrase, "Man's first mistake—the wheel," [1] characterizes a basic trend in human endeavor that has been directed throughout the ages toward a steadily increasing replacement of personal effort by mechanical devices. Modern Western civilization represents the climax of these tendencies by combining maximum technical perfection of labor-saving gadgets with their maximal mass distribution. Ostensibly, the United States of America are leading in these respects. What the general lack of exercise, such as walking, running, and carrying, has done to the muscular fitness of the motorized and TV-sitting young generation of this country has been drastically demonstrated by the extensive statistical studies of Hans Kraus and associates.[2] They revealed an alarming physical inferiority of American children, as compared with their counterparts in Austria, Switzerland, and Italy, and prompted the President to initiate the planning of nationwide corrective measures.

Interested advocates of more gadget manufacturing and of resulting further human immobilization may argue that there is no serious reason to oppose such a program, since muscular function has become largely superfluous in our way of living and loafing. However, regardless of what view one may adopt on this aspect, there remains the widely ignored fact that, if not the gastrocnemius or the biceps, one unique muscle, the myocardium, cannot be put safely to permanent relative rest and thus made incapable of coping with acute demands to which even the most civilized physical loafer is occasionally exposed.

Since antiquity, physically lazy, overfed persons of the social upper crust have been popularly known to be candidates for ailments of at least partially cardiac origin—shortness of breath, palpitations, chest pain, and the concluding fatal "heart attack"; but only in recent years has the problem of physical inactivity as a potential contributory pathogenic factor in cardiac mass degeneration begun to be systematically investigated.[3-5]

The most impressive data so far obtained are those of J. N. Morris,[3,4] whose large statistics among British workers suggest a reversed proportion between the degree of physical activity on the one hand and mortality from coronary heart disease on the other. Hairdressers and office clerks furnish the highest quota of cardiac deaths, whereas heavy agricultural and industrial laborers appear least afflicted. All in all, the incidence of death from coronary heart disease among "light" workers was more than double that among "heavy" workers. On a smaller scale, observations with similar implications were made by Luongo,[6] in California, and by Austrian insurance organizations.[7] It may also be significant that in Denmark and Holland, where the bicycle and not the automobile is the typical national means of transportation, the cardiac death rate is considerably lower than in the United States, despite an about equally high fat consumption.[8]

Obviously, coronary sclerosis on the one hand and degenerative myocardial damage on the other, although frequently coinciding and causally related, are essentially different pathological phenomena, and it is impossible

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at this time to evaluate clearly the degree to which physical inactivity contributes to the development of either one per se. Nevertheless, despite this limitation, it is no longer necessary or useful to consider the influence of physical activity and inactivity on cardiovascular function and structure merely in terms of nebulous generalities. Much is already known concerning relations between muscular exercise and neurovegetative (adrenergic-cholinergic) action on the heart and concerning the role of the neurohormones in cardiovascular metabolic pathogenesis.[9,10]

Myocardial oxidative metabolism and energetic efficiency (percentile conversion of oxidative energy into mechanical work) are dominated by the interplay of the adrenosympathetic catecholamines (epinephrine from the adrenal medulla, arterenol from the sympathetic nerve terminals in the heart) and vagal acetylcholine.[9,10]

Sympathetic adrenergic preponderance, either absolute by virtue of excessive catecholamine discharges or relative by virtue of inadequate vagal-cholinergic counterregulation, constitutes a threat to myocardial functional and even structural integrity. Both catecholamines, if not sufficiently counteracted by acetylcholine, diminish myocardial efficiency and, by wasting oxygen in a disproportionate fashion, are capable of inducing severe potentially necrotizing myocardial hypoxia, especially if the coronary blood supply is impaired by coexisting atheromatosis. Conversely, cholinergic vagal preponderance improves the oxygen economy of the heart muscle and increases efficiency, thus creating a metabolically and dynamically favorable situation.

Systematically trained sportsmen, particularly those engaged in endurance athletics, such as long-distance running, cycling, swimming, and skiing, display, as a rule, a highly developed vagal tone: bradycardia at rest, relatively slight acceleration under stress, rapid subsequent deceleration, a long isometric contraction period, absence of "hypoxic" changes of the electrocardiogram during severe exertion, small stroke volume at rest, but a great augmentation of the stroke volume during exercise. An unusually powerful contractility has been observed even in those enlarged "athletes' hearts," which formerly used to be mistaken as being pathologically dilated.[11-13]

The above enumerated cholinergic manifestations differ sharply from the adrenergic traits that usually characterize the hearts of persons with established degenerative (hypertensive and/or arteriosclerotic) heart disease[14]: a tendency toward tachycardia, marked and prolonged cardiac acceleration in connection with even slight muscular effort, hypoxia electrocardiographic changes, inadequate cardiac output, and reduced energetic efficiency.[15] Indirect evidence of an adrenergic preponderance as contributing fundamentally to the syndromes of angina pectoris and chronic or acute congestive heart failure (pulmonary edema) is provided by the therapeutic effectiveness of antiadrenergic and vagotropic measures: sympathectomy, ganglionic blockade, catecholamine-inactivating antithyroid therapy, and vagal stimulation through carotid sinus pressure and morphine.[9,10] Abnormally high concentrations of epinephrine have been detected in the myocardium of persons who had died in acute congestive failure and/or after recent myocardial infarctions.[16]

In addition to the available criteria of a beneficially high vagal tone in athletes and to the statistical evidence of a pathogenic adrenergic preponderance in the "loafers' hearts" of our motorized nonexercising population, it has been shown by Taylor et al.[17] that only three weeks of complete bed rest (in healthy volunteers) produces a marked and prolonged preponderance of adrenergic cardiac manifestations. This is probably due to an inactivity-induced deterioration of cholinergic counterregulatory mechanisms. Studies of the intermediate cardiac metabolism (glycogen, phosphocreatine, ATP) in nonexercised versus trained rats revealed a superior maintenance of biochemical normalcy in the latter under

acute stress.[18] Reactions of the untrained animals were analogous to those elicited by epinephrine or sympathetic stimulation.

Preliminary comparative investigations have been carried out recently by me and my associates on 40 physically inactive and 40 active healthy young men to evaluate the status of their cardiac adrenergic-cholinergic equilibrium. Blood pressure, heart rate, electrocardiogram, and chronodynogram (measurement of total systole, isometric, and isotonic contraction periods) were recorded at rest and during infusion of epinephrine and arterenol, before and after elimination of vagal counterregulation, by means of atropine. In the inactive group, the responses indicated a smaller degree of vagal regulatory effectiveness. These tests will be expanded during the coming year on an epidemiological basis by examining physically highly conditioned mountain dwellers of the Austrian Alps * as a corollary to Hans Kraus' muscular fitness studies on Austrian children. In animals, the influence of muscular training (treadmill) on the myocardial enzyme system is being investigated.

The above-outlined concept of myocardial functioning injury due to an inactivity-induced deficiency of cholinergic counterregulation against adrenergic damage concerns primarily the immediate neurohormonal influences on the heart muscle cells. However, the vascular supply of the heart appears to be likewise subject to alterations connected with physical activity and inactivity, respectively. It has been reported that the capillary network of athletes' hearts is unusually richly developed,[19] and Eckstein [20] has recently observed an augmentation of the total coronary bed in exercised animals.

No data are yet available that would permit a direct evaluation of the role which physical inactivity may possibly play in the origin of coronary atherosclerosis. At this time, it can only be said that adrenergic effects on vascular tissue metabolism seem to contribute to atherogenesis to a much greater extent than is being generally appreciated. The arterial walls contain relatively large quantities of potentially injurious catecholamines, the tissue-necrotizing properties of which have long been known. The deposition of cholesterol in the intima is accelerated and intensified by epinephrine.[21,22] Catecholamine-phospholipid compounds seem to possess a particular affinity toward arterial tissue.[23] Thus, an inactivity-induced relative adrenergic preponderance may be tentatively considered as a hypothetical predisposing factor also in the process of coronary atherogenesis. Certainly, current tendencies to attribute coronary atheromatosis exclusively to dietary lipid and fat consumption per se disregards the importance of primary variations in the lipid receptiveness of the vascular walls under neurohormonal and hormonal influences. I do not fear to be personally biased against the present emphasis on dietary factors in atherogenesis, since for the past quarter century I myself have persistently advocated a reduction of dietary fat and cholesterol intake after having carried out in 1931 a world-wide inquiry regarding epidemiological relations between the consumption of animal fats and the incidence of arteriosclerosis.[9,24-26]

Somatic and psychic phenomena are no longer separable. If the degenerative features of "loafer's heart" are, at least in part, initiated by lack of muscular exercise, the psychic implications of the latter must not be left unnoticed. An all-embracing philosophy of both physical and mental take-it-easy-ism deprives human life of most of its higher values and incentives. It leads to unimaginative boredom and breeds addiction to cheap entertainment and to the various commercially available substitutes for mental stability and spiritual creativeness. Such tendencies, combined with the frantic striving for material "success" in a conformist competitive society add frustrations and

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anxieties whose cortical and hypothalmic mechanisms cannot remain without detrimental effects on the neurovegetative biochemical equilibrium in the cardiovascular system. Excessive fat consumption does the rest, and it was again R. Price [1] who tersely described the ominous fate of Western man by saying: "Once the wheel was invented, the delicatessen store was inevitable."

Attempts to recondition salvageable middle-aged Americans by establishing safe bicycle pathways, as initiated by Dr. P. D. White,[27] and by means of physical training programs [28] are under way, but it seems more than doubtful that the overcivilized and pampered Western world will ever by its own volition revert to the healthier arduous living habits of its forefathers. Jean Jacques Rousseau's plea, *Retournons à la nature!* will hardly be more successful today than it was in the 18th century. Thus, modern medicine may have to face the task of developing new, convenient, and inexpensive artificial remedies to restore our badly upset central nervous and peripheral neurovegetative equilibrium, if we want to escape further degeneration.

Summary

Statistical evidence and experimental observations suggest that lack of physical exercise contributes to the development of degenerative cardiac changes, possibly also to that of coronary atherosclerosis.

The underlying mechanism seems to consist of a deterioration of cholinergic vagal counterregulatory effectiveness against the injurious influence of uninhibited sympathetic-adrenergic activity upon myocardial oxygen economy and myocardial mechanical efficiency.

Avoidance of effort, a traditionally dominant goal of Western civilization, entails far-reaching social and emotional consequences that are reflected in neurovegetative functional patterns with adverse cardiovascular implications.

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On Death

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In talking or writing about death we have a superstitious foreboding, handed down as an organic sense from all our ancestors, that the mere mention of death is a bad omen. It is as though we were attending our own funeral. Physicians have this trait in aggravated form. Some thoughts on death may be introduced by comment on a recent book dealing with death.[1] This book has a strong and sweeping indictment of the medical profession, but some will consider it instead an indictment of the central characters of the book. The charge against the medical profession is that physicians are unwilling or unable to deal honestly and frankly with patients who have a disease which is the equivalent of a death sentence. On the other hand, the fact that death came as it did to Charles Werbenbaker reveals the dangers of a do-it-yourself attitude to disease. Though it is robust, cavalier, Hemingwayward, even foolish, in our modern age of medicine, such an attitude courts death earlier than need be. If Wertenbaker had had a careful examination early in his illness, the calamity which is the essence of the book could well have been averted.

The central theme of the story is how a man and his wife, who tells the tale, confronted a short illness, cancer of the colon, which was to kill him within a few weeks after it was identified. The fatal delay of the neglected illness contains a lesson lost on the author. The story had special poignance for me, stirring memory concealed in the slumbering embers of the past. I knew Charles Wertenbaker in the very casual way one knows legendary characters a few years older than he is in school. I followed in his wake (if not his footsteps) at Episcopal High School and the University of Virginia. His own experiences in these schools were described in his books: "Before They Were Men," "Peter the Drunk," and "Boojum."

Death is a theme which can never be completed. It can never be seen from the inside. We can think of our own death, but we wind up with a series of guesses. Lael Wertenbaker describes with the clinical detail of a case report the processes of trying to preserve human dignity and honor, with no secrets between man and wife, before the looming reality of death as one of the realities of life. Pain is another theme. The description and even the conception of another person's pain is always difficult. The best that anyone can do is to draw analogies with his own experience of pain, trying to estimate that of another. A step-by-step description of physical wreckage, the decreasing effects of narcotics, and the final stage where endurance was exhausted and the patient provided the final assistance in his death are told frankly and vividly. In my own view, the summit of nobility and dignity is impossible when the ethical and moral aspects of death are denied or overlooked.

No doubt the public will react with righteous indignation against physicians for what is described as their cowardly attitude in not telling the truth. This is a highly individual matter. The considerable majority of doctors of my acquaintance tell the patients the truth, though not necessarily all, abruptly, and at once. They provide, if legitimate, some ray of hope to sustain difficult days. Removing all hope drains off courage, even in the brave. By explaining the outlook and helping the patient face it rather than by

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Bill Castle, being one of the most alive persons I know, will not take it amiss that my only ready tribute on this occasion consists on some thoughts about death. I have tried to look at the problem without evasion and with the directness we have learned to admire as one of the many virtues of Bill Castle.

subterfuge and lying, we avoid a cowardly game of make-believe which, by pretending that death does not exist, robs life of much of its dignity.

It is true that many doctors are embarrassed by death. It is a nuisance and a blunder. Our reactions to it may be infantile. Certainly all physicians must do some soul-searching in this matter and not be in league with the empty pompousness of the mortician, that beautician and necroromancer of death, who, putting rouge on the cheek of the dead, does not remove the finality of death but merely cheapens it and robs it of dignity.

A number of physicians have addressed themselves to formal consideration of the problem of death and dying, more from the practical point of view of what one does when confronting death than from a comprehensive philosophic approach to the problem. The medical problems of death fall in two classes, that which concerns sudden death and that which deals with the slow process of dying of a chronic, slowly fatal disease. The matter of dying suddenly I have discussed in an essay entitled "Remarks on Sudden Death," with various categories of sudden death—(1) instant physiologic death, (2) death where the dying process takes from a few minutes up to a half hour, and (3) a great miscellany of what is classed as sudden death where it is unexpected rather than sudden. I quote:

> The very word *death* is repugnant to physician and layman alike, and all our fertile inventiveness has been used to find linguistic by-passes to avoid mentioning death outright. The plain fact of death is planted squarely before us to contemplate. Living men can form no intimate conception of death, the one fact which the testimony of experience brings nearest philosophic certainty. Sudden death, especially, has been feared and the collective feelings of immemorial time are epitomized in the Litany which begs for deliverance from it. In spite of its unpopularity the problem of sudden death is one which has intrigued me for a long time. It has been observed and studied but little. One might think it had merely academic interest. But today, methods of resuscitation have been perfected so that occasionally it is possible to restore heart beat and life to a heart which has stopped beating in a person who is apparently dead. The topic has utility if we can find prodromes. It has more than just academic interest.
>
> The oldest recording we have of sudden death probably is in the Homeric legends. There are several accounts of heroes who were struck down unexpectedly, and who died. It was the belief of those who described what happened that they had been assailed by the Olympian deities, the thunderbolt of Zeus, for some reason of revenge or disobedience or jealousy. This particular, unanticipated form of dying was attributed to the gods on Olympus. In *Plutarch's Lives* there is an account of someone questioning Julius Caesar concerning the most desirable manner of dying, and he says that form which is not anticipated, namely, sudden death, is most desirable. In the Middle Ages there was little concern with the form and manner of dying, since death generally was looked upon as a release and an opportunity to enter another world which was thought to be better. So it was only with the rise of pathologic anatomy that our thoughts about sudden death became better crystallized.

Montaigne has much to say about death along with many sharp comments on physicians in his "Essays." They need to be read *in extenso* or browsed in repeatedly rather than compressed in a single area and analyzed. Keats said:

> How astonishing (here I must premise that illness, as far as I can judge in so short a time, has relieved my mind of a load of deceptive thoughts and images, and makes me perceive things in a truer light),—how astonishingly does the chance of leaving the world impress a sense of its natural beauties upon us! Like poor Falstaff, though I do not 'babble,' I think of green fields; I muse with the greatest affection on every flower I have known from my infancy—their shapes and colours are as new to me as if I had just created them with a super-human fancy. It is because they are connected with the most thoughtless and the happiest moments of our lives.

Latham, more than 100 years ago, said:

> The way of death is often smoother than the path of life; and great bodily anguish (there is reason to believe) does not often enter largely into the process of dissolution.

Comforting words for doctor and layman alike are found in Osler's essay entitled "Science and Immortality":

> I have careful records of about 500 death beds studied particularly with reference to the modes of death and the sensations of the dying. The latter alone concern us here. Ninety suffered bodily pain or distress of one sort or another,

eleven showed mental apprehension, two positive terror; one expressed spiritual exaltation, one bitter remorse. The great majority gave no sign one way or the other like their birth, their death was a sleep and a forgetting.

Worcester dealt with the subject in his wise little book entitled "The Care of the Aged, the Dying, and the Dead." His views are extremely helpful. It would be well if not only physicians but laymen knew the subject matter of the book. There can be few statements at once more comforting and comprehensive than Ryle's elegant little essay "On Death and Dying." Zinsser, in his autobiographical thanatopsis entitled "As I Remember Him," deals with the problem of his own terminal illness. For him, as for Keats, it was a time of sharpening perception, of exquisite sensitivity to the physical world. Perhaps his views are expressed best in his sonnets written during the same period. Alvarez published an essay entitled "Care of the Dying," and in the same year Gavey published the Buxton Browne Prize Essay entitled "The Management of the 'Hopeless' Case." Both essays probe deep into the simple personal questions raised by the inevitability of death, the final aspect of chronic disease, and a review of some attitudes of doctors, patients, and poets. Parks Weber has approached the problem of historic attitudes toward death as exemplified by tombs, monuments, medallions, and other physical memorabilia—not bedside reading certainly but a scholarly collection of historic attitudes embodied in sculpture and other three-dimensional forms of art.

There can be no rigid set of rules for determining the action of physicians which would be appropriate in every dying patient. For those with deep religious convictions, death hardly constitutes a problem. Likewise those with a strong character and a firm conviction that death is the final and inevitable part of life, the great pattern of nature which permits youth to be renewed perennially with the reiterated hope that each new generation may improve upon its predecessors. It might be generalized that death is more readily accepted by older people than by the young, although obviously there are exceptions. How then does a good physician help a patient to face death and, accepting the ways of nature, to meet it? It is not done by all the busy paraphernalia of scientific medicine, keeping a vague shadow of life flickering when all hope is gone. If adjustment may be looked upon as the *summum bonum* of contemporary phychiatry, its failure is exhibited when this pattern of complete egocentricity breaks down utterly in the face of death. If man lives as a stranger in a lonely crowd, he dies utterly alone. Whereas his entry into the world is the first stage of the dissolution of an intimate partnership with his mother, his final departure is the ultimate in solitary procedures.

I cannot conceive of the practice of medicine in which there is any breach of absolute trust and confidence between patient and doctor. A good physician cannot lie to his patient. If the truth be bitter, he must help the patient face it. On the other hand, I could not bear to practice medicine if I felt obliged always to tell everything I know or think I know. Our fallability condones only legitimate reticence. Thus, in the last analysis, each person must be judged and dealt with as an individual person. This maxim should be followed in life as well as in death. In a materialistic age, in a society which is certainly not notable for individual bravery, independence of thought, or a philosophic attitude, it will not be possible to make a philosopher out of every man during a terminal illness. Perhaps Sir William Jenners' three essential qualities for the medical man will see us through such difficult times: "He must be honest, he must be dogmatic, and he must be kind."

With the public's naïve acceptance of scientific medicine as magic, as being able to do all things for all men, the notion of failure is repugnant, even though this failure is built into the nature of things. Like the law of gravity, it may be defied, but always with failure. I have often considered the remarkable one-sidedness of medical education

from the point of view of the doctor-patient relationship. Think of all the years that go into our efforts at making doctors and when we have become doctors all the efforts that must go into our continual study in order not only that we keep up but that we improve. Consider the opposite partner in the social transaction of medical practice. The patient not only is never educated in being a patient, but even those with the best general education, the most alert, are likely to have a fabric of misconceptions woven into their ideas of medicine. These become a torment when a person has to wear the hair shirt of misunderstood disease embodied in his own illness. Without being melodramatic or flippant, I should like to see somewhere in the educational pattern of our time a plan to educate and train people how to be patients, what may be the significance of illness for him, how to meet it, and whenever he can, defeat it, but to accept reality.

Death, the final frustration of the aims and purpose of the good physician, is a theme of speculation for the bold. Fear of death is a perennial cause of fear for the timid. It is a topic omitted from medical school curricula and avoided as a theme for scientific papers and general discussion among physicians. It has been dealt with more perceptively by poets and writers than by physicians. There are many books on the subject, some historical, some philosophical, some medicolegal, and some religious.

The general reluctance of contemporary physicians to contemplate the subject of death is a by-product of a completely materialistic philosophy, although death is faced with equanimity by the strong and the brave even in the absence of any philosophy at all. Doctors have had serious charges made by laymen of their inability to face the reality of death. This may lead to the most extravagant and ridiculous symbolic maneuvers aimed at keeping extant certain representative traces of life, while final and complete death is temporarily frustrated or thwarted. This is of no advantage to anyone. It may soothe the doctor's conscience, which is disturbed by a feeling of guilt. It does impress the family about to be bereaved, but not always favorably.

I have no profound observations or original thoughts about death as we encounter it in the practice of medicine. I have thought about it many times, discussed it on occasion, written articles about certain forms and manners of dying, and once a year I give a lecture to junior students dealing with death. The possibility that euthanasia will be established as a sensible method of determining who should die, and when, has probably received a mortal stroke from the concentration camps of Europe in the present and recent past, with their orgies of human sacrifice, casual torture, and crude experiment. To anyone who has intimate knowledge of the functionings of a committee, it seems unlikely that wisdom could ever be reached on this of all subjects by a committee or caucus. Left up to one person, even the wisest judge would be assailed by doubts which would place impossible demands on him. While the failure to develop such courts for quasivoluntary death sentences may in some sense be antisocial, the morals and ethics of our time will have to be rewritten before the physician could become a voluntary accessory in the death of his patients. Once by law or by custom a physician has this power, his usefulness to any patient would be reduced to the vanishing point. He may not be judge and executioner too.

Thus it is the lot of a doctor always to be defeated ultimately. His victories are at best reprieves. He can never finally conquer death. But the great terror of death is fear, and fear can be defeated. Not only small skirmishes but whole campaigns can be won in this cold war. If, as he needs must do, the physician accepts ultimate death as inevitable, if he relieves it of terror and pain, he has done all that should be expected and all he can do.

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Characteristics of Influenza Virus Infections Observed in St. Louis from 1943 to 1957

I. Relationship of Changes in Antigenic Composition of Influenza Viruses to Morbidity from Influenza

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The recent occurrence of extensive influenza epidemics in the Far East and the spread of the infection to this country and other parts of the world has aroused new interest in the epidemiology of this disease. A long period of freedom from serious influenza outbreaks has been interrupted by the appearance of a virus strain of sufficient virulence to produce a high morbidity in areas in which it appears, although the accompanying mortality rate fortunately remains low. The continuous investigations of the U. S. Armed Forces Commission on Acute Respiratory Disease and the Influenza Study Program of the World Health Organization prepared the background for an appraisal of the characteristics of the new virus strain very quickly after its isolation and allowed an estimate to be made of the danger it presents of causing extensive epidemics.

The Virus Laboratory of the Department of Internal Medicine of St. Louis University has been concerned with the study of influenza continuously since 1943 and is one of the cooperating laboratories in the World Health Organization Program. While many thorough studies of individual outbreaks of influenza are available in the literature, there is a paucity of reports of serological and virological studies of a given area extending from the present time back through the years before the World Health Organization influenza reports became available. This seems an opportune moment to present in some detail an analysis of the occurrence of influenza in a centrally located city of the United States, based on observations of cases in a University Hospital, clinical studies being supplemented by serological tests and virus isolations.

The patterns of changes in levels of antibodies against various strains of influenza virus secured by averaging the results of many individual tests from year to year aroused our interest in the possibility of using larger pools of serum as an adjunct in the study of the epidemiology of influenza. Beginning in 1951, we have collected pools of serum of various types in a systematic manner. The results of these studies have been published in abstract form in a number of communications in the program of the Central Society for Clinical Research.[1-5]

Additional data have been presented as a Scientific Exhibit at recent meetings of the American Medical Association.[6,7] It is now proposed to publish in some detail the picture of the epidemiology of influenza in the St. Louis area as revealed by the entire series of studies.

The present communication will describe the outbreaks of influenza in St. Louis from 1943 to 1957, with an analysis of the antigenic properties of the viruses involved in these outbreaks. A number of the viruses used in the study were isolated in our own laboratories, and other strains were secured from outside sources. This presentation is

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From the Miller Pulmonary Laboratory of the Department of Internal Medicine, St. Louis University School of Medicine.

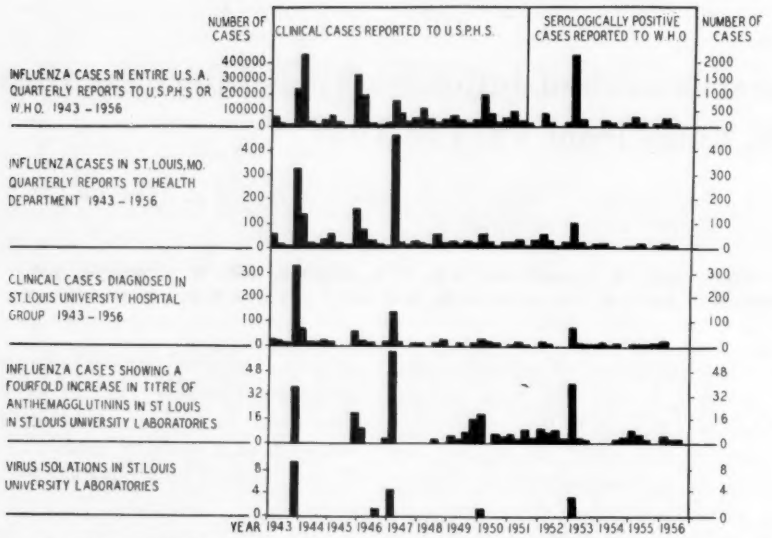

Chart 1.—Influenza in St. Louis, 1943-1956. Comparison of national and local public health statistics and clinical diagnoses with results of serological tests and virus isolations.

necessary to furnish a background for the understanding of the results secured by the study of serum pools to be described in other communications.

National and Local Incidence of Influenza Infections from 1943 to 1956

The extent to which a study of a centrally located area, such as St. Louis, gives information representative of the United States as a whole may be illustrated by statistics such as are presented in Chart 1. The incidence of reported cases of influenza by quarter years in the entire area of the United States is shown in the upper line of the chart. During the period from 1943 through 1951, these figures represent reports of clinical cases recorded in the United States Public Health Service Reports. From 1952 through 1956, the reports of serologically proven cases of influenza sent to the World Health Organization Influenza Study Program are graphically represented. The second line of the chart records quarterly reports of cases of influenza made by practicing physicians to the St. Louis Health Department. The third line represents clinical diagnoses of influenza recorded in the St. Louis University Hospitals. The fourth line illustrates the serologically positive cases of influenza secured in our laboratories regardless of virus type, and the fifth line records the virus isolations.

In general, the correlation between the occurrence of influenza locally in St. Louis and the national incidence is good. Of equal interest is the accuracy of clinical diagnoses of influenza in relation to the serologically proven cases. The multiplicity of recently recognized viruses causing respiratory infection of various types naturally has thrown doubt on the ability of the clinician to recognize an infection due to one virus. This difficulty remains serious and perhaps insurmountable in isolated sporadic cases. However, the short sharp outbreaks of illnesses of fairly constant pattern which are typical of influenza infection appear to be sufficiently characteristic to allow clinical recognition if an epidemic of any important proportions occurs.

Information regarding the general types of influenza virus responsible for outbreaks of the disease between 1943 and 1957 is presented in Chart 2. Clinical diagnoses of influenza made in the St. Louis University

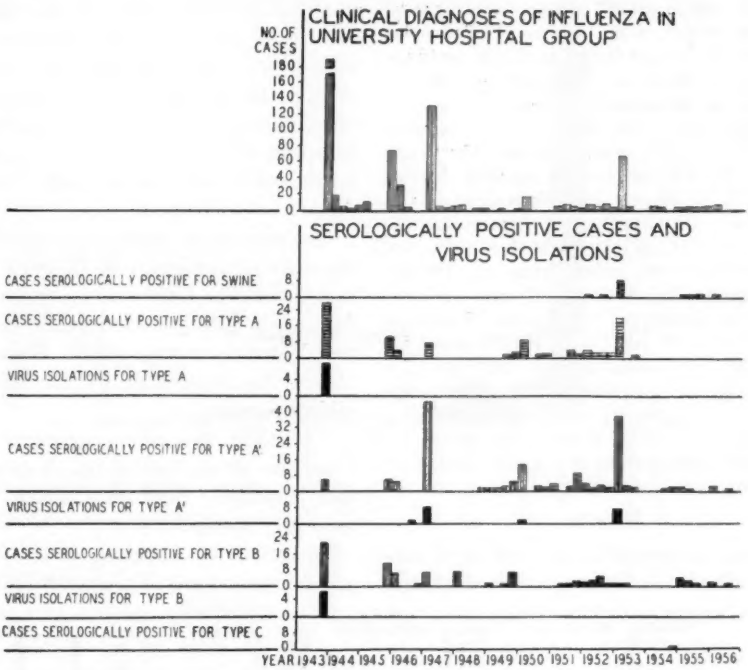

Chart 2.—Type of influenza virus responsible for outbreaks in St. Louis from 1943 to 1956 as revealed by serological tests and virus isolations.

Hospitals are shown in the upper line of this chart. Below are recorded serologically positive cases and virus isolations by quarter years for swine influenza, Type A, Type A′, Type B, and Type C, as obtained in observations made in our laboratories.

On the basis of virus isolation, there is evidence that Type A was involved in the epidemic of 1943. Type A′ viruses were isolated in 1946, 1947, 1950, and 1953, and serological evidence shows the presence of this virus type in 1957. Type B virus was isolated in 1943, and serological tests and virus isolations elsewhere suggest a preponderance of Type B cases in 1945, 1948, 1949, 1952, and 1954-1955. The data regarding swine and Type C virus presented here are too fragmentary to be of value.

Evolution of Antigenic Patterns of Influenza Virus Strains During the Period of the Study

One of the most interesting and important characteristics of the influenza group of viruses is the ability of a given strain to shift its antigenic character when exposed to adverse conditions in its environment. Slight changes in the character of prevalent viruses occur almost every year. After a period of years, these gradual changes may amount to a significant variation. A sudden marked shift in the antigenic character of a known group or the appearance of a new group of influenza viruses sometimes is seen, and these are considered to be the background of epidemics of considerable proportions.

In the study of these variations in our geographic area, we have compared the antigenic patterns of more than 20 strains of influenza virus.

Methods and Material

Chicken antiserum prepared against each strain were run against the strain used in their preparation and all other virus strains included in the study, employing the hemagglutinin-inhibiting test of Hirst. Results were expressed in percentage of antigenic relationship as calculated by the formula

of Archetti and Horsfall.[8] All sera used in these tests were treated with the receptor-destroying enzyme of vibreo cholera (R. D. E.) or periodate and heated to 60 C to eliminate nonspecific inhibitors of the hemagglutinin reaction.

The derivations of the strains of influenza virus included in this study were as follows. The strains of Swine, PR-8-A, and Lee-B virus were obtained from Dr. T. P. Magill. The Weiss-A strain was contributed by Dr. Carl Harford. Strains Norfolk-B-1952 and D.C.-B-1955, F.M.-1-A'-1947, and F.W.-1-A'-1950 were secured from Dr. Dorland Davis. Strain Denver-1-A'-1957 was obtained through the courtesy of Dr. Gordon Meiklejohn. Strains J.J.-C-1950 and Sendai-D-1953 and Asian virus grown exclusively in eggs and also after mouse passage were supplied by Dr. Keith Jensen from the U. S. Public Health Service Laboratories at Montgomery, Ala. The other 10 strains used in the study were isolated in our own laboratories in St. Louis.

Results

The results secured from the cross antihemagglutinin tests are graphically illustrated in Chart 3. Using 100% as the relation of each virus to itself, the antigenic resemblances of other strains are shown in columns of varying height in proportion to the strains with which they are compared, according to the results secured as previously mentioned by application of the formula of Archetti and Horsfall.[8]

The marked antigenic differences between the main virus Groups A, B, and C are well illustrated. The difference is shown between Strain PR-8-A, prevalent in the 1930's, and the Weiss-A strain, which Finland[9] has shown was responsible for the epidemic of 1943.

The shift in antigenic pattern between these two strains within the A group is far less, however, than that which occurred with the appearance of the A' viruses in 1946 and 1947. Antigenic variations among the A' viruses are illustrated by comparison

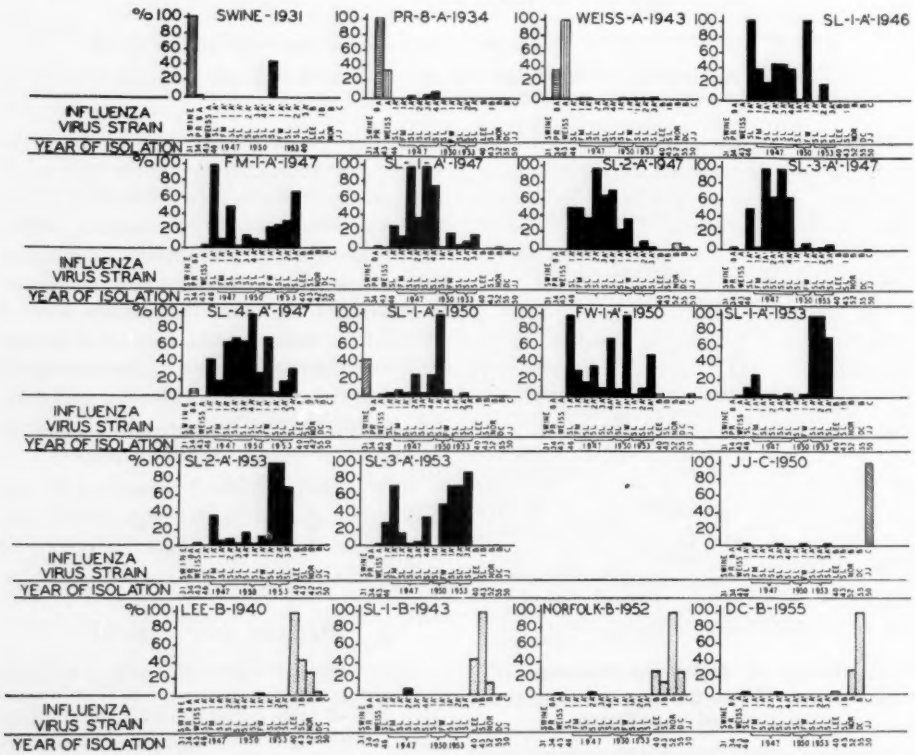

Chart 3.—Antigenic relationship of strains of influenza virus used in this study, expressed in per cent according to the formula of Archetti and Horsfall.

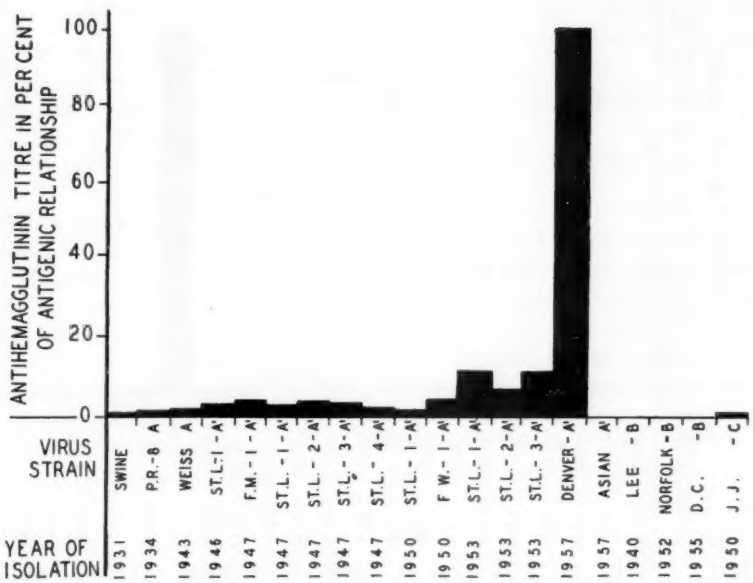

Chart 4.—Serological characteristics of influenza virus Denver-A'-1957 compared to 19 other strains, with use of the formula of Archetti and Horsfall.

of the patterns of the six strains of 1946-1947 A' virus, five of which were isolated in St. Louis, and the two strains of 1950 virus, one of which was isolated locally. Strain St. Louis-A'-1950 is particularly interesting because it appears more closely related to swine influenza than any other strain included in the study. A further shift away from the 1946-1947 pattern is seen in the three strains of 1953-A' virus, all isolated in St. Louis. The Denver A' virus, isolated by Meiklejohn in January, 1957, the antigen characteristics of which are shown in Chart 4, continues the evolution of these antigenic changes. It is most closely related to the 1953 A' viruses and shows progressively less relationship to the 1950 and the 1946-1947 A' virus groups. Obviously the antigenic changes become greater the wider the time interval between virus isolations.

A similar gradual shift in the antigenic characters is demonstrated in the Type B strains. This is shown by comparison of the Lee B strain isolated in 1940, a St. Louis strain isolated in 1943, and two strains isolated by Dr. Dorland Davis in 1952 and 1955, respectively. So little antigenic relationship exists between the 1940 and 1955 strains that a shift equivalent to that between A-PR-8-34 and the A' strains of 1947 has occurred.

The Sendai Strain, Type D Influenza of 1953

We have made some observations on the antigenic character of the Sendai Strain of Type D influenza, isolated in Japan, in 1953. Cross reactions were not found with any of the A, A', B, and C typing sera, with the exception of a very slight reaction with Weiss-A strain. A few persons and a number of our serum pools show very low titers of antibodies against this virus strain. We have no instance of the examination of paired sera which would suggest that this virus has been prevalent in the St. Louis area up to this time.

Characteristics of the Asian Virus Strain

Greatest interest at this time is centered on the Asian strain, already known to be responsible for extensive epidemics in China,

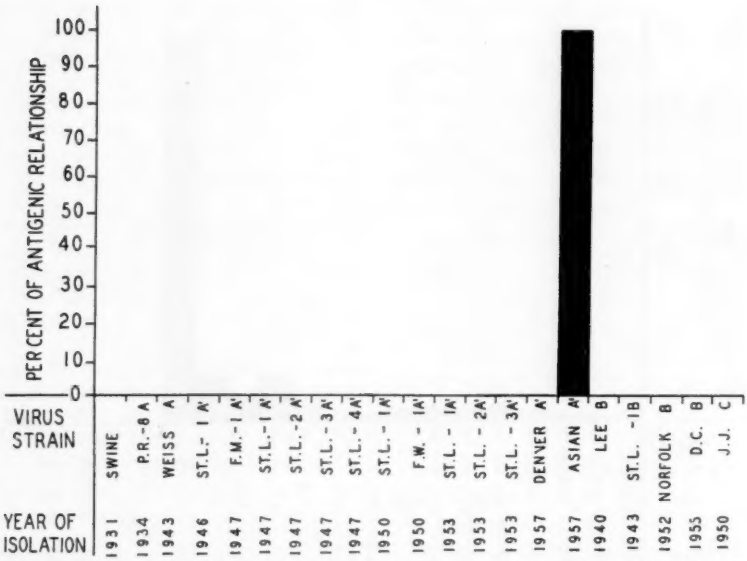

Chart 5.—Serological characteristics of influenza virus Asian A'-1957, cultivated exclusively in eggs, in comparison with 20 other strains, with use of the formula of Archetti and Horsfall.

Malaya, India, and the Philippines and a number of local outbreaks in this country. We have received this virus from Dr. Keith Jensen in two forms, a strain cultivated only in eggs and the same strain passed through four transfers in mice and then passed back into eggs.

Studies were made with the strain grown exclusively in eggs which indicate that antisera prepared against influenza virus strains previously mentioned in this study exhibit no hemagglutinin-inhibiting (H. I.) power against this strain. Antiserum prepared against the Asian strain is practically without action against A, A', B, and C virus strains.

With one exception, sera from individual persons and serum pools, collected in the St. Louis area have failed to show antibodies against the egg-cultivated strain. The exception is the serum of a Negro man, born in 1877 in Mississippi, with no evidence of respiratory infection or exposure to the Asian virus.

The Asian strain passed through mice is more reactive. Tested by the H. I. reaction with antisera of the other strains used in this study, there is at least some cross reaction with the Weiss-A strain of 1943. This strain has been passed through mice more recently than any of the other strains used in these investigations.

Tested against individual sera and serum pools, low titers of antibody against the mouse-passage Asian strain have been found in some instances, and fairly high titers, in some persons over 60 years of age. Up to Sept. 1, 1957, no virus isolations of the Asian strain have been secured in the St. Louis area. In examining paired sera from cases of febrile illness with symptoms resembling influenza, we have in no instance found a clear-cut fourfold increase in antibody titer between acute and convalescent serum specimens either by the H. I. test or the complement-fixation reaction.

Our studies of the Asian virus strain would indicate that it is of an antigenic pattern so different from previously isolated strains that the potential resistance of the population to the strain, in all probability, is low.

Comment

During the period of this study, from 1943 and up to Sept. 1, 1957, St. Louis experienced four epidemics of influenza of some proportions, namely, those occurring in the years 1943, 1945-1946, 1947, and 1953 (Chart 1). Several other very minor outbreaks were noted. The virulence of the virus strains as measured by effect on mortality was quite low. This would appear to be the picture typical of gradual evolution of influenza virus strains, permitting some carry over of immunity from related strains as the population becomes exposed to the newly developing variants.

Sudden marked shifts in antigenic characteristics would be expected to be accompanied by outbreaks with a high attack rate in the population.

The morbidity from influenza in the United States as a whole and in the St. Louis area illustrated in Chart 1 does not show the exact relationship that might be expected to result from the shifts in antigenic pattern as illustrated in Chart 3. For example, there was lesser shift in antigenic pattern between the PR-8-A virus prevalent in the 1930's and the Weiss virus considered to be the causative agent of the epidemic of 1943 than between these two viruses and the A′ viruses of 1946-1947. Nevertheless, the epidemic of 1943 had a much higher attack rate than the epidemic of 1947.

The appearance of St.L.-A′-1950, with considerable antigenic resemblance to swine influenza, was not marked by an appreciable outbreak of influenza in St. Louis but only by a slight increase in sporadic cases. Many believe that swine influenza, or a modification of this virus, was the cause of the pandemic of 1918. The antibody level found in the local population against swine and St.L.-A′-1950 just prior to the time that this virus was isolated was quite low. Hence, the result of the appearance of this variant was not as great as might be expected. It is true there is some cross relationship of this virus strain and the 1946-1947 A′ viruses, which probably were still prevalent in the area and hence may have afforded stimulation of the resistance mechanisms of the population.

Evidence can be shown that Strain D.C.-B-1955, isolated in Washington, D. C., by Dr. Dorland Davis, has appeared in St. Louis. Its antigenic pattern is radically different from that of the Lee-B virus of 1940. However, it shows considerable resemblance to the Norfolk-B-1952 virus, which appeared also in this area. There was no recognizable clinical outbreak of influenza in St. Louis at the time this virus appeared.

The results of this study show that resistance factors in human beings other than those demonstrated by the antihemagglutinin reaction must be taken into account in assessing the attack rate to be expected from a given virus strain. Changes in antigenic patterns shown by the H. I. test do not allow an accurate prediction of expected morbidity from influenza. Virus-neutralizing antibodies might be a better measure of resistance to infection. An unknown factor of virus strain virulence also may be involved. Nevertheless, the changes from the antigenic pattern of previously isolated strains in the case of the Asian virus are so radical that in this instance they should serve as a forewarning of what is likely to be an extensive outbreak of influenza. This prediction is strengthened by the experience of the populations of the areas in which the virus already has been prevalent.

Summary

A study of the influenza virus outbreaks in the vicinity of St. Louis during the past 14 years shows close correlation between the local and the national morbidity from this infection, whether this is determined by clinical diagnoses or serological and virological studies.

A comparison of more than 20 strains of influenza virus isolated at various times shows the frequent and progressive changes in the antigenic patterns which occur.

The degree of change in antigenic patterns as shown by the antihemagglutinination test is not a reliable index of the degree

of morbidity to be expected from a given virus strain in any one area.

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Effects on Blood Sugar of a New Potent Hypoglycemic Compound

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It has long been known that some diguanidine compounds, such as Synthalin A and B, produce hypoglycemia in man [1] and in laboratory animals.[2,3] In a systematic search for orally effective hypoglycemic compounds, Ungar et al.[4] found that phenethylformamidinyliminourea, a condensed diguanidine (or a diguanide), was effective. The compound has been called DBI by others,[4,6] but since the diguanide group is presumably essential for the hypoglycemic action, we have used the term phenethyldiguanide (PEDG) (Fig. 1).

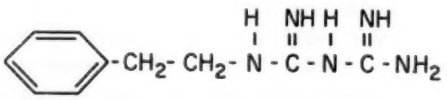

Fig. 1.—Phenethyldiguanide (PEDG).

These studies are a part of our efforts to define and elucidate the actions of this compound. The effects of PEDG on blood sugar in intact and eviscerated animals, on the hepatic glucose output, and on the degradation of insulin I^{131} were investigated.

Experimental Studies

Synthalin A, even in very small doses, is toxic and produces histological changes in the liver, kidneys, and pancreas.[3] A study of the toxicity of PEDG was done because of its structural and pharmacological [7,8] similarity to Synthalin. Twenty baby guinea pigs were divided into two equal groups. Ten animals received PEDG, 10 mg. per kilogram, subcutaneously twice daily, and the other ten received an equal volume of saline by the same route. At the end of six weeks there was no difference in the weights of the animals in the two groups; the ratio of organ weight to body weight was the same in both groups, and no histological changes could be seen in the liver, kidney, spleen, or pancreas. Ungar et al.[4] found no histological changes in the livers of animals given 20 mg. per kilogram per day by mouth for two weeks.

The compound was found to be effective orally, intramuscularly, intraperitoneally, and subcutaneously, but since the latter route gave the most consistent response, it was used routinely. Guinea pigs were used, since they were found to be relatively more sensitive to the hypoglycemic action of PEDG than were other common laboratory animals. Blood glucose was determined by the method of Somogyi.[9]

The response threshold was abrupt; 15 mg. per kilogram subcutaneously produced little or no hypoglycemia, whereas 20 mg. per kilogram produced a marked response by 2 hours, killing approximately 50% of the animals within 4 to 12 hours, presumably by hypoglycemia (Fig. 2).

Effect on Hepatic Vein Glucose Concentration

In an effort to delineate the site of action, the inferior vena cava, hepatic vein, and portal vein glucose concentrations were measured after PEDG administration.

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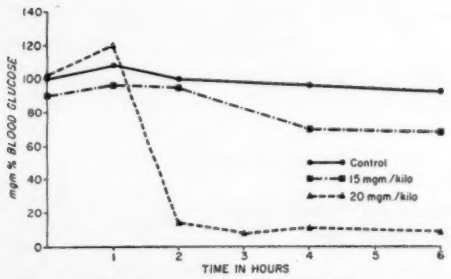

Fig. 2.—Effect of subcutaneous PEDG on blood sugar in intact fasted guinea pigs (mean values—eight animals each group).

Guinea pigs weighing between 300 and 400 gm. were fasted overnight. Dextrose, 0.5 gm. per kilogram, in 7.5% aqueous solution was given subcutaneously, and 30 minutes later PEDG, 30 mg. per kilogram, was given by the same route. Control animals were given injections of an equal volume of saline. The animals were anesthetized with ether. At time intervals of 30, 60, 90, and 120 minutes after the animals had received PEDG (or saline), the inferior vena cava was occluded just above the renal veins and blood was drawn caudal to the occlusion. Immediately thereafter, the inferior vena cava was occluded at the diaphragm, and blood was drawn from that segment of the inferior vena cava lying between the two clamps. This we assumed was hepatic venous blood. Portal vein blood was then withdrawn. These procedures took less than two minutes. At least six animals were killed at each time interval, and the blood glucose values represent averages (Fig. 3).

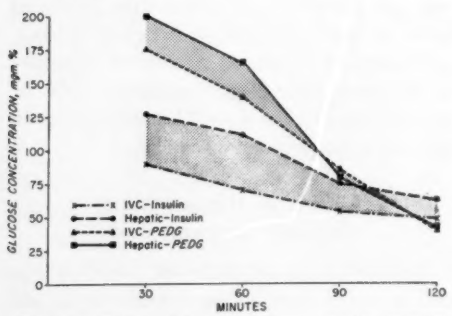

Fig. 4.—Effect of insulin compared with PEDG on hepatic vein and inferior vena cava glucose concentration.

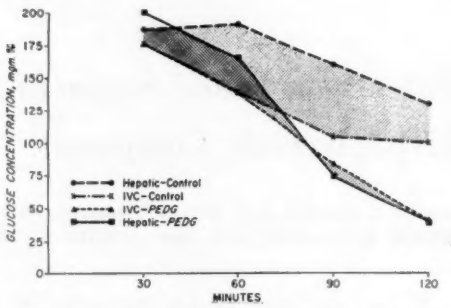

Fig. 3.—Effect of PEDG on hepatic vein and inferior vena cava glucose concentration.

Results

Figure 3 shows that in control animals the glucose concentration in the hepatic vein was higher than that in the vena cava at all of the time intervals. In the treated animals, however, the hepatic vein concentration fell more rapidly than did that of the vena cava, so that by 90 minutes the hepatic vein level was as low as or lower than that in the vena cava, suggesting a marked reduction in hepatic glucose output.

When hypoglycemia was produced by 1.1 units of insulin (Fig. 4), this effect on hepatic glucose output was not seen, although a comparable degree of hypoglycemia was produced.

It was not possible to overcome the hypoglycemia of PEDG by giving the animals 50μg. of epinephrine or 20μg. of glucagon intramuscularly 75 minutes after administration of PEDG and 15 minutes before withdrawing blood. Furthermore, these drugs had no effect on hepatic glucose output in PEDG-treated animals (Fig. 5).

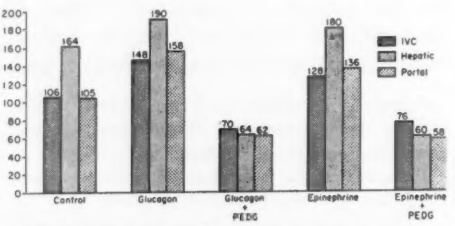

Fig. 5.—Effect of glucagon and epinephrine on PEDG-induced hypoglycemia (mean values—minimum of eight animals per group).

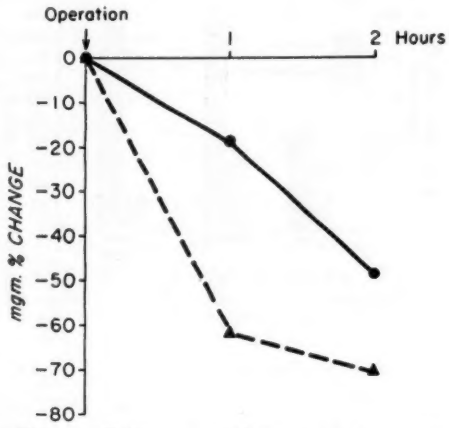

Fig. 6.—Effect of PEDG on "eviscerated" animals.

Effects in "Hepatectomized" Animals

To elucidate further the site of action of the compound, the following experiments were done on partially eviscerated animals. Sixteen fasted guinea pigs were anesthetized with pentobarbital (Nembutal) by intraperitoneal injection. A mass ligature was passed around the esophagus, the portal vein, the common duct, the hepatic artery, and the mesenteric vessels. The entire intestine, with the exception of about 2 cm. of distal colon, was then removed, and with this, the pancreas and spleen. PEDG, 20 mg. per kilogram was then given subcutaneously; an equal volume of saline was given the controls. Blood was drawn by cardiac puncture at one-hour intervals.

Results.—As shown in Figure 6, the operation alone produced a mean fall in blood sugar of 19 mg. per 100 cc. at one hour and 48 mg. at two hours in the control animals. In the treated animals, this fall was more pronounced: 62 mg. per 100 cc. in one hour and 71 mg. at two hours.

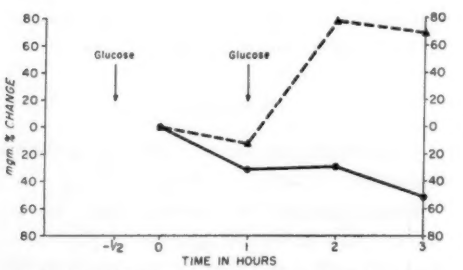

Fig. 7.—Effect of PEDG on blood sugar in "eviscerated" animals.

To avoid the confusion of the hypoglycemia produced by the operation itself, the procedure was done on 32 animals that had received dextrose, 0.5 gm. per kilogram subcutaneously 30 minutes prior to the operation. PEDG, 30 mg. per kilogram, was then given subcutaneously, and an equal volume of saline was given to the controls. A second, equal, dose of dextrose was given immediately following the one-hour blood withdrawal. Dextrose-loading (Fig. 7) abolished the hypoglycemia produced by the operation; there was a mean rise of 79 mg. per 100 cc. after the second dose of dextrose. In the treated animals, however, there was definite hypoglycemia of −28 mg. per 100 cc. at two hours and −54 mg. at three hours.

Effect on Insulin Degradation

PEDG was shown by Williams et al.[10] to inhibit, in vitro, the degradation of insulin I^{131}. This property was studied in vivo by the following technique. Sixteen male guinea pigs, weighing 300-400 gm., fasted 17 hours, were given PEDG, 20 mg. per kilogram subcutaneously. An equal number of control animals were given saline. Ninety minutes after injection of PEDG, insulin I^{131} (0.5 cc., 0.11 units, 12μc.), previously dialyzed against phosphate buffer at pH 7.4, was injected into the jugular vein with use of ether anesthesia. Thirty minutes after insulin administration, the animals were again anesthetized and exsanguinated by having blood withdrawn from the aorta. Liver and plasma were treated with trichloroacetic acid (TCA), as previously described.[11] Urine was aspirated from the bladder, the penis having been ligated at the time of administration of the labeled insulin, and all samples were counted in a well-type gamma counter.

Results.—As shown in Figure 8, there was a greater amount of radioactivity in the TCA supernatant of both plasma and liver

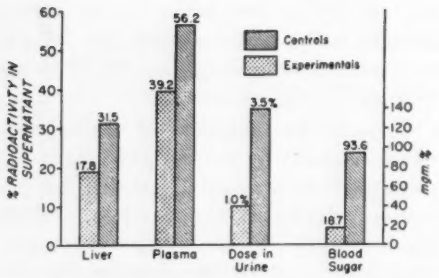

Fig. 8.—Effect of PEDG (20 mg. per kilogram) on degradation of insulin I^{131} (average values).

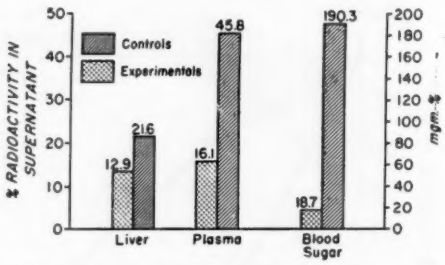

Fig. 9.—Effect of PEDG (20 mg. per kilogram) on degradation of insulin I^{131} (nephrectomized animals, average values).

in the control group. The urine of the controls contained a greater percentage of the administered dose. Since radioactivity in the TCA supernatant and that found in the urine presumably represented degraded insulin,[11] these results suggested that PEDG inhibited insulin degradation. Furthermore, the amount of total radioactivity (supernatant and precipitable) is the same in PEDG-treated and in untreated groups when expressed in terms of the total dosage administered per unit of weight of tissue [11]; therefore, a distributional phenomenon cannot explain the apparent inhibition of degradation.

Nephrectomy alone inhibits the degradation of insulin I^{131}.[12] Tyberghein [8] has shown that glomerular filtration is significantly reduced following PEDG administration. Therefore, the degradation studies were repeated, using 16 guinea pigs in which the renal vessels were ligated immediately prior to PEDG (or, in the controls, saline) injection. The results (Fig. 9) are virtually the same as in the intact animals, although there is a greater fall in blood sugar values.

Comment

PEDG in doses of 20 mg. per kilogram subcutaneously produced striking hypoglycemia in guinea pigs. Although the hypoglycemia was accompanied by a marked fall in hepatic glucose output, hypoglycemia was also produced in the absence of functioning liver. Tyberghein and Williams [13] have shown a striking decrease in liver glycogen content following administration of PEDG. Thus, while there is a distinct effect on hepatic carbohydrate metabolism, this action alone does not explain the hypoglycemic action of PEDG. It should be mentioned that Ungar et al.[4] found no change in hepatic glycogen content following administration of PEDG. However, the oral dose used by these investigators was found, in our hands, to be too small to have any hypoglycemic effect.

Since the hypoglycemic action was still present in the absence of the intestine and pancreas, the effect cannot be due to a decreased absorption from the intestine nor can it be due to the stimulation of insulin secretion.

Although PEDG inhibits the degradation of insulin I^{131}, the action of insulin on liver glycogen and on hepatic glucose output is so different from that of PEDG that it is doubtful if insulin "sparing" plays a significant role in the production of hypoglycemia by PEDG.

Although we have shown that this compound acts unlike insulin and unlike the sulfonylureas, the precise site and mechanism of action await further studies.

Summary

Phenethyldiguanide (PEDG) is a potent hypoglycemic agent in the guinea pig.

Hypoglycemia is accompanied by a marked fall in hepatic glucose output; however, hypoglycemia can be produced in the eviscerated animal.

The degradation of insulin I^{131} is inhibited by prior administration of PEDG, but it is not felt that this action is of significance in the production of hypoglycemia.

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Thirst—The Acquisition of Water

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A century ago, Claude Bernard [1] pointed out that the cells of most higher organisms do not really come into direct contact with the external environment but exist in their own internal sea—the extracellular fluid. Subsequently [2] he deduced from a relatively few facts that it was the stability of this milieu intérieur which constituted the "primary condition for freedom and independence of existence."

In recent years much attention has been given to the role of the kidney in regulating the volume and osmolality of the body fluids. Nevertheless, it must not be overlooked that the kidney can subserve this office only by excreting excesses—it cannot furnish water or solutes to replace deficits. Hence, an adequate intake of water is of primary importance for body fluid homeostasis in man, since inevitable losses occur continually, both through renal and extrarenal channels.

Under usual circumstances water deficiency manifests itself promptly by thirst, the satisfaction of which relieves the deficit. The role of such voluntary regulation in maintaining the dynamic steady state is so well known that it is frequently disregarded in the pursuit of mechanisms by which equilibrium is reestablished after voluntary regulation has been prevented or is ineffective. Thus the usual immediate response of man to a fall in environmental temperature is the donning of more clothing and the seeking of warmer surroundings. Provided these can be accomplished, cutaneous vasoconstriction and shivering may be unnecessary for heat conservation. Similar phenomena abound throughout the biologic world. The earthworm seeks moist soil; the kangaroo rat remains in its burrow during the heat of the desert day; the turbellarian flatworm, *Gunda ulvae*, remains in the narrow range of the stream below the high mark of the spring tides and above the low of the neap tides. The obscurity of the driving forces which lead to these reactions does not detract from their fundamental importance as regulatory devices.

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From the Medical Service and Research Laboratory, Boston Veterans' Administration Hospital, the Department of Medicine, Boston University School of Medicine and the Department of Medicine, Tufts University School of Medicine.

Mr. Theodore A. Phillips, Manager of the Medical Book Department of Little, Brown & Co., Boston, gave permission to extract material for this article from my book, "Body Water in Man: The Acquisition and Maintenance of the Body Fluids," 1957.

Thirst—What Is It?

Thirst has been known to man throughout the ages and has been discussed scientifically for over two centuries.[3] Nevertheless, details as to its mechanism remain obscure—and even the definition of the word is not agreed upon. Some would limit the term to states of water deficit; others would insist that a state is not one of thirst unless it is relieved by water ingestion. Many would include in its definition the localization of sensations in the mouth and pharynx. Certainly, dryness of the oral passages is a common concomitant of water deficiency. But dryness of the mouth may also result from the action of drugs or of disorders of the salivary glands. Under these conditions water ingestion gives but transient relief. That dryness of the mouth and the sensation of thirst may arise from psychologic factors is also well known. In fact, at one time this phenomenon was employed to determine the guilt or innocence of a suspect. If he could eat a bowl of dry rice it was apparent that his conscience was clear and

his salivary flow adequate![4] A sensation believed by the sufferer to be thirst may occur in the absence of dryness of the oral mucosa and in fact in the presence of excess water in the body.

For the purposes of this discussion, Brillat-Savarin's definition, in 1825,[5] seems entirely clear and operationally adequate: *La soif est le sentiment intérieur du besoin du boire.* (Thirst is the inner consciousness of the need to drink.) Bellows[6] has stated this, "Thirst may be defined as the urge to drink water."

The complexities of the problem of thirst may be illustrated by studies of various authors who have studied dogs with esophageal and gastric fistulae. They have noted that the act of swallowing may allay the urge to drink, despite the fact that no water enters the stomach; so, too, may distention of the stomach by a balloon, although the original stimulus of an actual water deficit or of hypertonicity remains.

Bellows[6] induced the urge to drink in dogs with esophageal fistulae either by water deprivation or by the intravenous infusion of a 20% salt solution. As soon as access to water was permitted, the dogs commenced drinking, swallowing amounts far in excess of those needed to correct the dehydration or hypertonicity. This water, of course, could not enter the stomach because of the fistula. Nevertheless the urge to drink ceased, at least for a period of time. Thus the act of drinking may allay thirst despite a continuing water deficit. Contrariwise, if an amount of water sufficient to correct the deficit is directly introduced into the stomach without the act of swallowing or wetting of the pharynx, there is no immediate effect on the urge to drink, although the amount "swallowed" is diminished. This is in contrast to the intact animal, which stops drinking as soon as the amount of water entering the stomach approximates the amount of the deficit. However, when the water is introduced directly into the stomach and access to water not permitted for 15 minutes (that is, until there is time to allow for some absorption and correction of dehydration), the urge to drink disappears.

Distention of the stomach by a balloon inserted through a gastric fistula does not abolish the urge to drink following the intravenous infusion of strong salt solution but reduces by more than one-half the usual volume of water swallowed by such dogs consequent to a similar infusion when the stomach has not been distended.[7]

Thus—in the dog at least—the act of swallowing, the state of distention of the stomach, and the volume and concentration of the body fluids may all be involved in the urge to drink.

Pure Water Deficiency

In man the sense of thirst may be evoked by a variety of factors, including those consequent to social and psychologic conditioning as well as those involving body fluid volume deficits and body fluid hypertonicity. It may be noted at once that pure water deficiency has two effects: hypertonicity of both the extra- and intracellular fluid and a diminished volume of both these fluids. Since the two phenomena are inseparable in pure water deficiency, delineation of their respective roles in causing thirst depends upon observations made on subjects who, by chance or experimental design, undergo hypertonicity without body fluid volume reduction or the converse. The only known way to accomplish the former is through the administration of a solute which is so freely diffusible across cell membranes that it causes no shift of water. Observations have been made concerning a number of such substances. The ingestion of hypertonic urea by man, although raising the osmotic pressure of both the extracellular and intracellular fluid, does not appear to stimulate thirst until after it has produced diuresis with resultant reduction in body fluid volume.[8] Although the blood urea level in both acute and chronic renal failure may reach levels such that the total osmotic pressure is increased by 100 mOsm. per liter or more, provided there is no diuresis, thirst has not

been observed by me to be a necessary concomitant. In the dog, administration of hypertonic urea or isomannide solutions leads either to no drinking or to the drinking of no more than half the quantity of water ingested following the administration of equally hypertonic salt solutions.[6,9,10] The ingestion of a mole (46 gm.) of alcohol may raise total body fluid tonicity by 20 mOsm. per liter. Nevertheless, the subjects of Strauss, Rosenbaum, and Nelson,[11] when they drank 50 gm. of alcohol rapidly, did not experience thirst until after diuresis had ensued. To be sure, the alcohol may conceivably have dulled the perception of thirst for water!

Thus, although it is quite clear that pure water deficiency, leading both to hypertonicity and a contracted volume of the body fluids, results in thirst, the evidence at hand suggests that the hypertonicity is not the major stimulus of this sensation.

Sodium Chloride Depletion

Depletion of the normal body content of salt leads at first to simple contraction of the volume of extracellular fluid. Later, if access to water has been afforded, hypotonicity of the body fluids ensues.

The "thirst" which may be associated with experimental sodium depletion has been commented upon by McCance[12]: "We have all noticed a peculiar sensation in the mouth, which is present all day long. This is not thirst, although Miss Edwards (one of the subjects) considered that it was so, and drank freely in her attempts to obtain relief." Another subject noted that he was "thirsty all morning—drank a lot, but water seems to make little difference."[13] McCance also notes that the "thirst" so often complained of by patients with intestinal obstruction may be brought about by salt deficiency and may often be relieved by small amounts of hypertonic saline. I can attest that the "thirst" experienced several days following an abdominal operation was unrelieved by drinking large amounts of water but was rapidly assuaged by a cup of highly salted bouillon. After paracentesis in patients with hepatic cirrhosis whose serum sodium level and osmolality may already be depressed, a sense of thirst can lead to the voluntary ingestion of large quantities of water and thus lead to further lowering of serum sodium and osmolality. In such patients it is obvious that the removal of isotonic ascitic fluid cannot affect the osmotic pressure of the remainder of the body fluid, although it patently does affect its total volume and compartmental distribution.[14] Although McCance considered that the sensation which led to the drinking of water was not thirst, certainly his fellow subjects of depletion thought it to be. A rather similar phenomenon occurs in the experimentally salt-depleted dog, leading to a very considerable increase in water drinking and consequent polyuria. Whether or not the dogs are "thirsty," they nevertheless may double their previous water ingestion. Such depletion has been produced[15,16] either by dialysis or by inducing diuresis with 50% sucrose and maintaining the animals with a diet containing no more than a milliequivalent or two of sodium and chloride daily. The results were the same in either case. The thiocyanate space (an index of extracellular fluid volume) and the Evans blue dye (T-1824) space (an index of plasma volume) decreased 10% to 20%, and serum sodium and chloride concentrations fell. Rises in serum protein level and hematocrit value reflected the estimated decline in plasma volume. As long as the animals were maintained in the depleted state they consistently drank as much as twice their previous water intake and excreted large volumes of urine in consequence. As soon as they received salt in amount sufficient to reexpand their depleted extracellular fluid volume, water ingestion and urine output declined to the control levels. In view of the fact that the polydipsia occurred despite serum sodium levels as low as 125 mEq. per liter and serum chloride levels of 75 mEq. per liter, it is apparent that hypertonicity was not the stimulus for drinking.

Nadal, Pedersen, and Maddock [17] produced extracellular fluid depletion by means of intestinal suction in persons who received adequate amounts of water to prevent general dehydration. Despite the fact that a state approaching circulatory collapse appeared imminent, thirst was not a complaint of the subjects. No explanation for this discrepant observation is at hand.

It would thus appear that the urge to drink is a frequent, if not invariable, result, in both man and dog, of a contracted volume of extracellular fluid consequent to salt depletion and that this occurs despite hypotonicity of the body fluids.

Pure Extracellular Fluid Volume Deficiency

Thirst may occur in a variety of situations in which there is reason to believe that the volume of the extracellular fluid is contracted without concomitant change in osmolality. Hemorrhage, for example, is widely accepted as a cause of thirst,[4,18,19] and it is traditional that blood donors be given fluid after venesection. Nevertheless, Holmes and Montgomery [20] found that "blood bank donors showed no thirst and no drop in salivary flow, unless there was an associated circulatory collapse and a drop in blood pressure and marked vasoconstriction." They also studied the responses of dogs to bleeding of 10% to 40% of their blood volume.[21] No drinking was observed in any instance. When hemorrhage was combined with the intravenous infusion of a 20% solution of sodium chloride the drinking pattern did not differ from that obtaining when saline was infused without concomitant bleeding.

At the moment the question of the relationship of blood loss and thirst in man must be held *sub judice*. It may well be that if the amount of bleeding is not excessive and the subject well hydrated at the time, thirst does not occur, and the very absence of circulatory phenomena may serve as an index of an adequate extracellular fluid volume. Further controlled studies are sorely needed.

The thirst accompanying diarrhea is so well known as to require little comment. Latta, in 1832,[22] repeatedly uses the adjective "burning" in referring to the thirst of malignant cholera. Since the gastrointestinal fluids are virtually isotonic with the extracellular fluid, their loss should not alter the osmolality of the body fluids. However, the ingestion of plain water in response to the dreadful thirst of cholera—particularly since urinary suppression was so common in the malignant form—should indeed have led to the lowering of the "neutral saline ingredients" of the blood originally noted by O'Shaughnessy in 1831.[23] It was his analyses of the blood in cholera patients which stimulated Latta to employ the intravenous infusion of saline solution in such patients. This would appear to be the first recorded instance of intravenous replacement therapy. Latta notes that its use abated thirst or, when given early enough, prevented its appearance. He infused as much as 330 oz. (10 liters) into one patient within a 12-hour period and no doubt saved her life. It may be noted that he utilized a silver cannula and "Read's patent enema syringe" to accomplish the infusion.

Thirst has been observed by me as a premonitory manifestation of impending diarrhea before any fluid has actually left the body. It is likely that the sequestration of isotonic fluid in the lumen of the intestine serves to reduce the effective extracellular fluid volume.

The common custom of imbibing fluid at mealtimes may or may not derive from thirst. Observations by Gregersen [19] on dogs are of interest:

> Continuous records obtained under normal laboratory conditions show that dogs rarely drink except when they are fed: when food is withheld, they drink only about one-fourth of the usual amount. The need for water after meals is explained by the temporary withdrawal of body fluids for the formation of digestive juices. Since these secretions are reabsorbed in the later stages of digestion, the desire for water should also dis-

appear. That this is the case may be shown by withholding water for six or eight hours after a meal and then measuring the water drunk, at which time the dog consumes much less water than it usually drinks during the postprandial period when given water *ad libitum*.

Thirst may be an early manifestation of cardiac failure. During the accumulation of edema fluid, but before it becomes clinically evident, patients may note excessive thirst.[20] The suggestion offered is that the transudation of fluid in the more dependent parts of the body leads to a diminution in effective circulating blood volume and consequent thirst.

Drinking Patterns

As already mentioned, social and psychologic factors may be intimately involved in the sense of thirst in man; thirst is an interpretation of subjective sensations—an "inner consciousness." Therefore, thirst per se can never be studied in animals. Drinking patterns may be the result of "thirst" in animals, but this we can never know. Certainly man's drinking patterns with regard to alcoholic beverages, tea, and coffee are probably not primarily the result of thirst in the sense that is being used in this discussion.

One of the most interesting observations concerns the drinking patterns of different animals following the same stimulus—dehydration. The burro, cat, dog, and rabbit relieve their entire deficit by gulping water as rapidly as swallowing permits.[24] The camel and the donkey behave similarly, being capable of drinking, within minutes, a volume of water equivalent to more than 30% of their dehydrated body weights.[25] (The advantage to a steppe animal, like the donkey, in being able to replete water loss within 70 to 80 seconds has been pointed out, since predators are likely to be waiting at water holes.) The guinea pig, hamster, and rat (like man) punctuate their drinking.[24] However, when the dog is stimulated to drink by an intravenous infusion of hypertonic sodium chloride solution there is an extreme degree of variation in individual animals. Some complete their drinking very promptly or at least achieve the major share of it within five minute. Others take small amounts at frequent intervals for an hour or more. Still others wait 5 to 10 minutes after water has been offered before commencing to drink. In contrast to the individual variation among different animals is the remarkably consistent and reproducible pattern in the same animal when subjected to the same stimulus on different occasions.[10]

Another fascinating observation concerns the "metering" of the volume drunk by animals. In response to dehydration they ingest approximately the correct quantity to make up their deficit. "In no instance is water ingested voluntarily in an excess sufficient to give rise to water diuresis."[24] However attuned the dog may be to a more or less natural stimulus to drinking, he is less competent to judge the amount he must drink to dilute injected hypertonic saline solution to isotonicity. Di Salvo[26] has noted that following the infusion of 20% sodium chloride solution at hourly intervals the amount of water ingested is sufficient generally to dilute the salt to a 1.8% solution. However, no measurement was made of salt excretion during the studies, which extended over several hours. With more dilute solutions[27] that increase the volume of water in the body to a significant degree, there may be no voluntary drinking. This was the rule with 1.5% to 4.0% saline and was frequently observed even with stronger solutions. Nevertheless, the consistency of response of individual dogs is striking. Thus some regularly drink far too little; others, repeatedly too much, while a small group invariably approximate the correct amount.[10] This consistency of response to the same stimulus in individual dogs has been of considerable value in assessing responses to varying stimuli.

Contraction of Intracellular Fluid Volume Associated with Expanded Extracellular Fluid Volume

When the tonicity of the body fluids is increased by the administration of a strong

solution of a solute that remains largely extracellular—such as hypertonic sodium chloride—extracellular fluid volume is increased both by the volume of fluid administered and by the shift of water out of the cells in response to the osmotic gradient. Intracellular fluid volume is accordingly contracted just as in pure water deprivation. Thirst results, as noted by Leschke, in 1918,[28] after he had rapidly injected 10 to 20 cc. of 20% to 25% sodium chloride solution into patients; he reported that they complained regularly about "the sudden appearance of such severe thirst that one had to give them large quantities of water to drink immediately."

The experiments of Gilman[9] were the first clearly to separate the effects of elevating the osmolality of the body fluids by a freely diffusible solute, such as urea, which does not alter the compartmental distribution of these fluids, from those produced by a solute such as sodium chloride, which, remaining largely extracellular, expands the volume of this compartment while at the same time contracting the volume of the intracellular fluid. Gilman demonstrated that the latter maneuver was a far more effective stimulus to drinking in the dog than was the former, despite the fact that each produced an equal increase in the osmotic pressure of the blood serum.

Dogs were infused intravenously with 2.5 cc. per kilogram of body weight of either 20% sodium chloride or 40% urea solution (in either case an approximately two-thirds osmolal solution). The osmotic pressure of the serum rose equally with the two solutions. However, the specific gravity of the serum, largely a measure of serum protein concentration, fell with the saline infusion, indicating a shift of water from the cells to the extracellular fluid. After the infusion of urea, which enters cells freely, serum specific gravity remained unchanged. After the infusion of strong salt the animals drank an average of 42 cc. per kilogram of body weight of water within an hour, thus restoring their osmolality virtually to normal. After the urea infusion they drank less than half as much water, so that the osmolality of their body fluids was still elevated at the end of six hours.

Bellows,[6] employing dogs with an esophageal fistula, noted that sham drinking commenced immediately after an infusion of strong salt solution, but only after a 10- to 15-minute delay when the infusion was of urea of equal osmolality. He further noted that the rate of drinking was halved in the urea studies as compared to those employing sodium chloride.

Holmes and Gregersen[10,29] confirmed and extended these observations. They found that hypertonic urea and isomannide, neither of which produced internal fluid shifts, led to water drinking varying from nil to one-half as much as followed the infusion of equally hypertonic solutions of sodium chloride, sulfate, or acetate, which remained extracellular and hence led to a movement of water out of the cells. Sorbitol in 50% concentration produced changes similar to saline solutions. Dextrose, however, in 50% or even 75% solution led to less than half as much drinking as sorbitol. Initially it produced the same expansion of thiocyanate and Evans blue dye spaces as did the sodium or sorbitol solutions, but at 50 minutes these had decreased to less than control values.

It may be pointed out that thirst has not been reported consequent to 50% dextrose infusion in man, nor did a spontaneous complaint of thirst follow the intravenous infusion of 1 ml. per kilogram of body weight of a 50% galactose solution in a small series of observations[30] Why these two sugars, which should produce immediate changes in intracellular fluid volume similar to those induced by salt and sorbitol solutions, fail to stimulate thirst remains unexplained.

It would thus appear that although raising the osmotic pressure of the body fluids without change in their volume may at times lead to some thirst, when such hypertonicity is associated with a contraction of the

volume of intracellular fluid, thirst inevitably ensues.

The Role of Salivary Secretion

That dryness of the mouth—whether due to dehydration, mouth-breathing or the administration of atropine and related compounds—may lead to a sense of thirst is well known. Whether the time-honored practice of holding pebbles in the mouth when on the march and without water is really effective in relieving thirst has not been documented, but excellent relief of thirst has been noted as a result of sucking a lemon.[31] Holding water in the mouth was less effective.

Lepidi-Chioti and Fubini, in 1885,[32] anesthetized with cocaine the back of the mouth of a boy who presumably had diabetes insipidus and were able to relieve thirst for from 15 to 35 minutes. Rinsing the mouth with water gave only a few minutes' relief. However, Weir, Larson, and Rowntree[33] found that neither anesthetization of the mouth with cocaine nor the injection of pilocarpine (to stimulate salivary secretion) would relieve the thirst of four patients with diabetes insipidus temporarily deprived of water. An extensive discussion concerning the role of salivary secretion in thirst will be found in Cannon[4] and in Gregersen.[19] The following quotation is from Gregersen:

> Putting all the above facts together, we arrive at the conclusion that the decrease in plasma volume caused by depletion of the water content of the body is significant as the first step in the physiological mechanism of thirst which signals the need for an increase in the water intake. A dry mouth is therefore an important physical sign of a lowered blood volume, a fact which the surgeon has inferred from his practical experience; namely that a patient with a dry mouth is a poor surgical risk and cannot withstand hemorrhage as well as the patient whose tongue is moist and glistening.

The Neutral Integration of Thirst

The area in the brain involved in the integration of the various stimuli which lead to the "inner consciousness of the need to drink" has not been delimited for man. That such an area may exist and may be located in an area near the supraoptico-hypophyseal system is suggested by two patients with both diabetes insipidus and some evidence of anterior pituitary insufficiency. One, a woman, voluntarily drank 3 to 4 liters of fluid daily.[34] Her polyuria, however, was of such magnitude that this intake was insufficient to prevent her serum sodium level from rising to between 150 and 165 mEq. per liter. In spite of this hypertonicity, she did not experience thirst or increase her drinking. When vasopressin (Pitressin) was administered, urine volume declined but fluid intake remained unaltered. Serum sodium and chloride values decreased to within the normal range. Upon omission of vasopressin, polyuria recurred but drinking habits remained unchanged and hypertonicity reappeared. It would thus appear that in this patient, who suffered from a brain lesion in the hypothalamicohypophyseal area, hypertonicity of the body fluids did not serve as an effective stimulus to thirst nor did it lead to an increased water intake.

The second such patient, with diabetes insipidus and evidence of some anterior pituitary failure resulting from a presumed eosinophilic granuloma, when she was given large solute loads, developed alarmingly high serum electrolyte levels although permitted water ad libitum. The suggestion was made[35] that the failure to ingest sufficient water to correct hypertonicity was due to damage to the "thirst center."

Although cases such as the above are no doubt quite rare, another type of disturbance in the regulation of fluid intake is not uncommon. Even if the stimulus to thirst and the integrating mechanism are intact, unless there is consciousness thirst cannot be perceived and unless there is access to water, and strength to obtain it, regulation cannot be achieved. Numerous instances have been recorded of severe dehydration and hypertonicity in patients whose state of consciousness varied from moderate clouding to frank coma. Many of these patients were so ill and so weak that even if they per-

ceived thirst they could neither complain about it nor obtain water for themselves. Such cases have been reported by numerous observers.[36-43] Many of the patients, in addition to their inability to obtain water or their unawareness of its need, had a greater than usual water requirement because of fever, sweating, diarrhea, vomiting, or polyuria—the last due to the increased amount of nitrogen requiring excretion because of the catabolic reaction to trauma or a high protein-and-solute tube-feeding formula or both—and in some instances they had an inability to excrete a concentrated urine, either because of renal disease or concomitant diabetes insipidus.

For example, a patient receiving daily 2 liters of a gavage formula containing 8% protein and 0.5% sodium chloride will have a minimum of 25.6 gm. of nitrogen and 10 gm. of sodium chloride for excretion. If all the nitrogen is assumed to be excreted as urea this will constitute 914 mOsm. which with approximately 342 mOsm. of sodium chloride will total 1256 mOsm., an amount which a man with normal renal function can excrete in 1 liter of urine. However, if there is such damage to the kidneys that the maximum achievable concentration of solutes in the urine is 700 mOsm. per liter, then a minimum urine volume of 1800 ml. would be required. Assuming a normal insensible loss of 1000 ml. through skin and lungs and disregarding stool and sweat loss and metabolic water gain, as well as other solutes demanding water for excretion, such a patient would sustain a net loss of 800 ml. of water daily.

An area in the brain involved in thirst has been sought in animal experiments. Bellows and Van Wagenen [44] produced injury to the hypothalamicohypophyseal area in dogs, employing a transbuccal approach. Subsequent histologic study of the lesions was not reported. Twenty-three of an unknown total number of operations led to diabetes insipidus—temporary in twelve, permanent in the remainder. Adiposogenital dystrophy was also a common sequela. From various studies, none of which included determinations of serum or urine osmolality or electrolyte concentration, they suggested that the polydipsia encountered was primary and due to the brain lesion, rather than secondary and due to the diabetes insipidus. Although the data are insufficient for one to be certain, it may well be that this was the case in at least certain of the animals.

Stevenson, Welt, and Orloff [45] produced bilateral electrolytic lesions in the ventromedian hypothalamic nuclei of rats. When fed a wet-meal diet or when fasted but allowed access to water, these animals exhibited no abnormalities in water metabolism. However, if given a dry diet, although allowed free access to water, they failed to drink enough to prevent chronic dehydration and elevated serum sodium levels. The same workers, with Wall (quoted by Stevenson, Welt, and Orloff [45]) have observed primary hyperdipsia in monkeys after prefrontal lobotomy.

Witt and his associates [46] noted that electrocoagulation of the ventral extent of the anterior hypothalamus in dogs led to well-defined and sustained polydipsia. When the coagulation was extended dorsally and laterally, there was temporary or permanent absence of thirst. This was evidenced by a complete lack of interest in water, even to the point of critical dehydration and death; yet, when the water was "baited" slightly with milk or meat juice, the dogs "drank it ravenously, i. e. as food but not as water." Posterior pituitary injection (Pituitrin) and forced hydration were necessary otherwise to continue the dogs in satisfactory condition, "since thirst continued to be absent in the presence of physiological stability maintained by therapy."

Andersson's studies in goats present the strongest evidence available today for the thesis that there is a specific hypothalamic area involved in the thirst mechanism and that this is in close approximation to the receptor mechanism for antidiuretic hormone excretion. A needle of 0.5 mm. diameter was inserted through the cranial vault

to the hypothalamus and so secured as to remain in a fixed position, the goat's horns serving as a convenient shielding device.[47] Localization of the point of injection or electrical stimulation was accomplished both by x-ray and histologic examination after killing. Access to water was permitted throughout. Within 30 to 60 seconds after the injection, through the previously placed needle, of 0.003 to 0.01 ml. of 2% to 3% sodium chloride solution, the goats commenced to drink. This they continued more or less constantly until they had imbibed between 2 and 8 liters of water in two to five minutes.[48] This effect was not always repeatable and could be obtained at most three times in succession. Obvious diuresis started about one hour after the first injection and the attendant water drinking.[47] When 0.8% to 0.9% sodium chloride solution was injected there was no effect, although subsequent injection of a 2% solution produced the response.[49] These results of course afford no real proof for the existence of cells reacting specifically to osmotic stimulation, since 2% sodium chloride solution could have a general stimulating effect on nerve cells. When the injection was subsequently found at histologic examination to have been made into the third ventricle, or visibly had broken through the ventricular wall, the extent of drinking was less pronounced but the stimulation was more easily repeated.[49] The maximum amount of water drunk after one injection of 0.1 ml. of 2% sodium chloride solution was 9 liters.

When electrical stimulation (0.5 to 1.5 volts, 60 cps) was employed, the goats commenced drinking after a latent period of 6 to 45 seconds and continued for 3 to 6 seconds after the current was shut off.[50] This was repeatable at will, and in one instance a goat drank an amount of water equivalent to 40% of its body weight, with resultant marked hemodilution and lowering of serum electrolytes. Hemoglobinuria occurred.

The authors point out [48] that the goat is a very satisfactory animal to study, since the large rumen permits the storage of extremely large volumes of fluid before there occurs distention of the remainder of the gastrointestinal tract (which inhibits drinking in other animals).

Electrical stimulation in the anterior but not in the caudal portion of the "drinking area" in an animal undergoing diuresis led to antidiuresis and milk ejection similar to that encountered following the intravenous injection of posterior pituitary extract.[48,50]

The precise area in which stimulation is successful lies between the descending fornix column and the mammillothalamic tract at the horizontal level of the middle of the hypothalamus.

Preliminary experiments, performed on dogs in which the "drinking area" was electrocoagulated by the Hess technique, led to partial or complete loss of thirst although diabetes insipidus did not ensue.[50]

Greer [51] was not primarily interested in thirst or drinking when he studied hypothalamic stimulation in the rat. Nevertheless, it seems unquestionable that in 1 of the 25 animals the electrode placement led to polydipsia upon electrical stimulation. Anatomic study after death showed that the noninsulated tips of the electrode were immediately behind the level of the paraventricular nuclei and were at the lateral edge of the dorsomedian nuclei and in the area between the latter and the ventromedian nuclei. The paraventricular and supraoptic nuclei were not involved. The electrodes were connected by insulated wire with a nondirectional pick-up loop placed subcutaneously between the scapulae. On the day following electrode placement, stimulation was commenced with an automatic device which caused a current—estimated to be between 0.5 and 1.0 volt—to pass between the electrodes for five minutes every half hour. The source was a 60 cps square-wave D. C. pulse of 2 msec. duration. Immediately after the commencing of this stimulation the rat drank water avidly or, if water was not available, licked the walls of the cage. When 5% sodium chloride was substituted for tap

water, the rat would commence drinking but quickly withdraw from the container. Nevertheless, the urge to drink was so strong that the rat would return repeatedly during stimulation and lap up more of the strong salt solution. Given free access to tap water, it drank in excess of 400 ml. per day. The response was diminished on the fourth day and could not be elicited on the following day.

Gilbert [52] electrocoagulated the midbrain in rats at the level of the subcommissural organ. When the procedure led to destruction of this organ there was an immediate, drastic, and persisting fall in water consumption by the animals. However, when extracts of the organ were injected into normal rats there was a decline rather than a rise in water intake. This was interpreted by Gilbert to be the result of overcompensation by the animal's own intact subcommissural gland antagonizing the tendency of the injection to increase water consumption.

Summary—Thirst as a Regulatory Mechanism

Thirst is an important regulating mechanism of body fluid volume and osmolality. Stimuli to thirst include cellular dehydration (that is, a decreased intracellular volume of water) as well as extracellular volume deficits (interstitial or plasma volume or both). Presumably integrated in the hypothalamic region in the brain, these stimuli produce the consciousness of a need to drink, provided there is no disturbance in the integrating mechanism and consciousness is unclouded. If water is available and the person capable of obtaining and ingesting it, a deficit of water in the body will not occur and the role of the kidney will be limited to the disposal of excesses. Adolph [24] has stated the case in animals and proposed the following hypothesis:

Regulation of body content of water, therefore, is accomplished not merely by the overflow of any excesses. Equally it is adjusted by measured intakes. Drinking is just as reproducible as excretion, and water balance is approached with equal accuracy from both sides. In no instance is water ingested voluntarily in an excess sufficient to give rise to water diuresis, [This is obviously not true for man, whose drinking habits often lead to water diuresis.] and in no instance is water excreted in a diuresis that gives rise to water drinking. The 2 regulatory responses are quantitatively compatible; what could be more probable than that they are connected to a common detector of body water content?

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Scientific Method and Social Role in Medicine and Psychiatry

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It is a great pleasure and a privilege to join with Dr. Castle's friends, colleagues, and former students in celebrating his 60th birthday by contributing to a *Festschrift* in his honor.

Although the note which I will introduce stems from my work in psychiatry and psychoanalysis, I believe that it is nevertheless fitting for the occasion. I propose to consider the similarities and differences between medicine and psychiatry. This subject is usually debated in informal gatherings in a personal and impressionistic fashion. Instead of this approach, my purpose here is to present a scientific inquiry into this subject, meaning thereby an examination of medicine and psychiatry as bodies of knowledge, methods of treatment, and professional roles in society.

The Common-Sense Approach to Medicine and Psychiatry

I shall begin with what may be called the common-sense approach to medicine and psychiatry. According to this view, medicine comprises a very large area of inquiry and practice and embodies all the sciences and techniques that contribute to our understanding of human health and the cure of disease. Psychiatry, viewed as the study and treatment of "mental health and illness," belongs, therefore, to medicine and is a branch of it. This view of medicine has contributed, no doubt, to the formation of the notion of "psychosomatic medicine." This area is regarded as lying in the borderland between medicine (proper) and psychiatry, and the concepts and techniques of both disciplines are thought to be particularly pertinent to it.* The common-sense view of the relationship between medicine and psychiatry finds expression in two fundamental spheres of our daily life: in medical education and in the social structure of medical (including psychiatric) practice. It gains support, moreover, as is often the case, from the actual circumstances of our everyday life. In this instance, the social circumstances—the fact that psychiatry is practiced by "physicians," for example—are themselves reflections, at least in large part, of the phenomena that I propose to scrutinize. This "obvious" relationship between medicine and psychiatry may be likened to the equally obvious everyday impression that we have of the earth as a flat surface. And, in fact, we do act as if the earth were flat when we build a house, a road or a bridge. The relevance of the earth's spherical shape arises only when we travel large distances and particularly when we remove ourselves (at least mentally) and think of standing somewhere outside in space. I present this analogy to emphasize the fact that we invariably have one picture of a situation when we are deeply

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* Note in this connection Professor Woodger's pertinent comments: "Words like 'psycho-somatic' are only invented to heal a breach which need never have been made. I have maintained that medicine has no need for a dualistic language (in the usual sense) and would be better without one. If we use person language we can see that medicine is involved in the sociology of family life and that the so-called medical psychology deals with problems arising from the difficulties of living together under one roof of persons related by the parental or some derived relation" (Reference 13, pp. 317-318).

involved in it and another when we take a more distant contemplative attitude toward it. For instance, too, a war is one thing to a harassed foot-soldier, another to a historian; an epidemic of poliomyelitis is one thing to an anxious parent and quite another to a microbiologist in a distant community, and so forth. To get another perspective of the relationship of medicine and psychiatry to each other, and to other sciences, it is necessary therefore for us to leave our accustomed observation posts and look at the matter from the "outside." Before doing so, I want to reiterate the point that the common-sense view of medicine and psychiatry is generally comforting and often useful, in a practical sense. It is inadequate, however, from the viewpoint of scientific accuracy. Finally, in this, as in many other situations, it is sometimes necessary to have both a "common-sense" and a "scientific" view of the matter at hand and to use one or the other as the need arises. It is well, however, not to mistake the former for the latter.

Medicine, Psychiatry, and the Philosophy of Science

In a general way the point of view toward medicine and psychiatry that I advocate is anything but new. It is, of course, basically the attitude and method of science: to search, to question, and to take nothing (or as little as possible) for granted. But there is more to it than this. For the subject matter that we are considering is itself complex, i. e., "medicine," "psychiatry," and their relationship to each other. These are not the raw materials of science, such as we encounter when studying falling bodies or red blood cells. They are rather themselves complex abstractions, scientific systems, and social bodies. In examining this sort of material it is nevertheless possible to take a scientific position. How this can be done has been shown by modern philosophers of science who have pursued fascinating and fruitful studies of the "science of science" (which goes by the name "philosophy of science" or "metascience"). This type of work will serve as a guide in this inquiry into the nature of medicine and psychiatry. Briefly, this entails an attempt to analyze and describe in operational terms, as far as possible, the characteristic features of these disciplines and of the relationship, if any, between them. If our endeavor is successful we will not have contributed—strictly speaking—to medicine or to psychiatry, but to the philosophy (in the sense of "metascience") of medicine and psychiatry.[12] This is not to say, however, that considerations such as these do not have the utmost relevance to both the practice and the theory of medicine and psychiatry.

Methods of Examination and Treatment in Medicine and Psychiatry.—In this inquiry I shall depend heavily on two key concepts: one is "scientific method," the other, "social role." Both are familiar concepts. Yet a brief statement of their meaning is warranted because in medicine, as the practical art of healing, these distinctions are often confused or forgotten. By scientific method is meant the particular technical procedures that are used. For example, the characteristic techniques of medicine are as follows: the so-called physical (*sic*) examination of the body (as if it could be examined any other way), the use of x-rays, laboratory tests (for diagnosis), and chemical substances or surgical operations (for treatment).† The

† I have deliberately omitted history-taking from among the typical techniques of medicine, for it introduces some very complicated methodological and philosophical considerations. Let us merely note that history-taking employs a psychological method, i. e., conversation with the patient. This method, however, may be employed either for simply obtaining a history of the body as a machine (this would be similar to the history of a rock or a tree) or for eliciting the history of a person (which may be likened to the history of a notable "historic persons," that is, biography), or for both. In the practice of medicine a "history" is taken, usually in the context of a medical (physicochemical) framework. A social or psychiatric "history," while using the same word, refers to a subject matter that is quite different. In actual practice, it is of course possible to have

(Footnote Continued Next Page)

emphasis is on the nature of the method, not on the physician's intentions (to diagnose, to cure, etc.). This is important because such things as "intentions" are essentially private; moreover, the actor-observer (physician) may be mistaken about his intentions, may be unconscious of them, or may even falsify them in a deliberately goal-seeking manner. In contrast, technical methods are public.[7] Any one who cares about them can see and decide for himself of what they consist. As Einstein[3] so beautifully stated it: "If you want to find out anything from the theoretical physicists about the methods they use, I advise you to stick closely to one principle: don't listen to their words, fix your attention on their deeds."

Let us apply this principle to medicine and psychiatry. We are generally told that anything which aims to "help sick people" is "medicine"; "medicine" (proper) is said to help people who are sick in body, "psychiatry," those who are sick in mind. We shall not belabor this Cartesian pseudosolution of a problem which is pressing both in its theoretical import and in its everyday bearing on medical practice. Instead, let us carry out Einstein's suggestion, and rather than listening to words let us scrutinize the deeds. Now, it is clear that medicine is concerned with the workings of the human (and animal) body as a physicochemical machine.[9] It endeavors to investigate how this machine is put together (anatomy), how it works (physiology), and, when it breaks down, how to repair it (therapeutics).

In doing this sort of work, the physician naturally uses the methods appropriate to the object of his study, and these are the methods of physics and chemistry. Indeed, adherence to this mode of operation has resulted in brilliant advances in modern medicine and has led to better understanding of the human machine as well as to greater know-how concerning the repair of its breakdowns.

Before sketching what psychiatrists do, I must specify the sort of psychiatrist I have in mind. This is necessary because the practice of some psychiatrists can be subsumed under the heading of medicine without significant modifications. This is so for those who consider psychiatry as that branch of medicine which deals with diseases of the brain. These men use physical methods (by and large), such as electroshock, drugs, and surgery, and accordingly their practices are indistinguishable (by this criterion) from those of their medical colleagues. Others, however, rely entirely on psychological (including social) methods of investigation and treatment, and it is to them that I have reference when I use the words "psychiatry" or "psychiatrist."[11]

Thus, the object of interest in medicine is the body (as a physical object), while in psychiatry it is the person as a "social being" or "person." The psychiatrist's methods are psychological and center around the process of communication, chiefly with verbal symbols. There is much variation and subtlety among the various psychological methods of investigation and therapy, ranging all the way from hypnosis and moral exhortation at one end of the spectrum to psychoanalysis at the other. For our present purpose specific details of technique are unimportant. What is important is merely to note the sharp difference that exists when the issue is sketched in this way, between medicine and psychiatry. From this perspective the respective objects of study are wholly different (patient as "body" versus patient as "social being") and the methods used are likewise different (physicochemical versus psychosocial techniques).

An objection may be raised against the sketches of medicine and psychiatry which I have drawn. It is true that medicine and psychiatry use different methods, but—it may be argued—their object of study and

various mixtures of the two types of histories. These considerations make it clear, finally, that whenever there is verbal communication between patient and physician, there arises a nonphysical (personal) dimension to the doctor-patient relationship which, in turn, makes adherence to a purely physicochemical frame of reference insufficient to encompass the totality of the "medical situation."

their aim are the same, namely, to investigate and to treat the "whole person." Medicine takes care of the body, psychiatry, of of the mind, and thus divided but reunited, the task can be carried to completion. There is something to be said for this contention, since as an abstract program it sounds reasonable and attractive. In actual practice, observation tells us that it does not work out in this way. To "take care" of a whole person, when ill, is often (though happily not always) more than any one person can do. And so psychiatrists have been criticized by their medical colleagues for neglecting the "organic" side of their patients' problems, and psychiatrists have countered by accusing nonpsychiatric physicians of neglecting to deal with their patients' personal troubles. This counteroffensive has been extended in recent years, so that many psychiatrists have become fond of believing that "doctors" are wont to treat their patient's "bodies" with surgical operations and drugs when, "at bottom," the patient's troubles are "really mental." Unfortunately, many thoughtful medical men have come to have doubts of their own about the large numbers of so-called "emotional disorders" which they have habitually treated with prescriptions and have come to believe in the verity of this psychiatric accusation. It is my impression that the problem with which we deal here is far more complicated than it appears and involves much more than merely a choice between physical and psychological "treatment." I shall comment on this matter further in the closing sections of this essay.

In addition to this cross criticism, medicine and psychiatry have developed another solution to this contemporary dilemma. It is a retreat to an aggrandizement of their own position and a belittling of that of their opponent. Medicine has pointed the way in this, but psychiatry has, unfortunately, bested its rival in this competitive game of self-approval. Their respective claims are, of course, well known. From the medical side comes the argument that only studies based on physics and chemistry are "scientific" and that those problems that have not yet yielded to this approach will do so in the course of time. Even Freud himself never quite gave up this idea.[5] ‡

Psychiatrists, unable to make an identical counterclaim, have instead taken recourse in their self-appointed role as the only "humane" practitioners of medicine on the modern social scene. Accordingly, the psychiatrist arrogates to himself the prescientific humane role of the physician as comforter of the sick. Neither claim can be supported by facts. Both patently serve to make their adherents feel better. Nor is the issue of "humanitarianism" really relevant in this context, for it would seem to me that in all situations in which the physical-medical approach is effective it is *ipso facto* "humane," and, similarly, when the psychological-medical approach is effective, it is "humane." [10]

In any case, self-aggrandizing claims deflect attention from certain problems inherent in the scientific methods and social roles of these disciplines, to which J. Robert Oppenheimer [6] called attention (though speaking about a subject not immediately related to our present concern) with the following words:

> There is much more that one might know than any of us are ever going to know. There is much more to know than any of us are ever going to catch up with; and this is not just the trivial fact that we don't work hard enough; it is not the trivial fact that things are difficult to learn. It is that any form of knowledge really precludes other forms; that any serious study of one thing cuts out some other part of your life. Narrowness is not an accident of one place, but it is a condition of knowledge.

It is in vain that the psychiatrist prides himself that he is interested in the "whole person," for he really is not, unless this

‡ See in this connection the Editor's Introduction to "Studies on Hysteria," in which it is stated that, "To the end of his life, however, Freud continued to adhere to the chemical aetiology of the 'actual' neuroses and to believe that a physical basis for all mental phenomena might ultimately be found" (Reference 1, p. xxiv).

high-sounding expression is simply used to denote the experiential and social facets of "man." The psychiatrist must neglect and must delegate to others the genetic, physiological, pharmacological, and other methods of studying and modifying the hypothetical "whole man." By the same token, it would be ridiculous, were it not tragic, for the physician to persevere in his belief that the physiochemical method is the only relevant approach in his work with patients as human beings, rather than as biological organisms. In speaking of this problem, Professor Woodger stated (Reference 13, p. 278 §):

> Neither can the method, which uses the physical language exclusively, deal with persons, except by means of the abstract notion of body. This method undoubtedly suffices for many purposes. But, as we shall see, it has serious disadvantages in medicine and it involves ignoring almost all to which we as persons, but not as scientists, attach most importance. This leads many people to contradict in their lives what they say in their laboratories.

Inquiry into some of the reasons that lead physicians and psychiatrists to espouse these irrational beliefs carry us logically to considerations of the notion of social roles and their relevance to problems of medical practice.

The Social Roles of Physician and Psychiatrist.‖—Social role is a concept taken from sociology. It denotes the part that a man plays in a complex social structure vis-a-vis other people with whom he comes in contact. One may also speak of a person's own concept of his social role and how this may or may not coincide with that assigned to him by other people. Some examples will illustrate what we have in mind.

In a smoothly functioning society or human situation, the participants are usually unaware of the "existence" of social roles. For example, it is the role of a watchmaker to repair watches, not refrigerators. This strikes us as trivial, but it is only one step removed to say that it is the social role of a patient to obey his physician. Yet many patients resent this role and in fact refuse to "play" it. So much in the way of introducing this concept. Let us now take a brief look at medicine and psychiatry with the aid of these constructs.

It is obvious that social roles pertain to particular locales and specified times. Thus the social role of medicine, and even more that of psychiatry, is very different today from what it was only a half century ago. Even at a specified period, such as at the present time, people in different parts of a large country, and those with varying cultural and educational backgrounds, will have different concepts of, for instance, the physician's role. In what follows I shall concentrate on the social roles of physician and psychiatrist, respectively, as these are perceived by physicians and psychiatrists themselves. The views of well-educated men generally will be very similar, while those of others may differ greatly.

The social role of medicine today seems to me roughly as follows. The physician is viewed as a scientific technician who repairs the human body. The material that is disordered is physical, "objective," and "real." These terms are important. I say that the disorder is physical to denote that it has anatomical and physiological correlates, that is, it is a fracture, an infection, a tumor, or a metabolic defect. It is objective, meaning that it is verifiable with the aid of certain testing methods and by other physicians (not by the patient). Anemia, for example, is "objectively" verifiable, whereas a phobia is not. Allied to the notion of "objective," is the notion of "real." A fractured hu-

§ Though keenly aware of the scope of the psychological methods of science, Woodger unfortunately uses the word "scientist" in the passage quoted clearly to refer only to the physical scientist qua scientist. Indeed, it is in the nature of things—If I may put it this way—that the scientist should behave differently in his laboratory than, say, in his home. There is no contradiction here. The trouble arises when the physician-scientist behaves in his consulting room, or at the sick bed, as if he were in his laboratory.

‖ Psychiatrists, of course, are physicians, according to ordinary verbal usage. In this context, however, I use the word "physician" to refer only to the nonpsychiatric ("organic") physician.

merus is objective; its pain is "real." Pains and other complaints which are not so correlated with bodily events tend to be viewed as not quite as "real." We thus come to the picture of the "real doctor." Real doctors are doctors of medicine; they treat real (bodily) diseases, and use real (surgical or pharmacological) treatments.¶ Implicitly, the role of the psychiatrist is defined by contrast: he is the doctor who does not behave like a "real doctor." The psychiatrist could thus be said to treat unreal (imaginary, nonbodily) diseases and to use unreal ("just talking") methods. I have deliberately used somewhat colloquial language to sketch these social roles, since it is easier to convey a vivid image—and this is after all what is needed here—in this way.

Some Practical Applications

I now want to comment briefly on some practical applications which may be derived from the foregoing considerations.

Implications for Medical Education.—First, let us consider the frequent suggestion that the psychological and social side of medicine should be given greater emphasis in medical school so that the physician shall be properly equipped to handle those patients who suffer from psychosocial disorders. I am in agreement with this position, though not so much for the alleged therapeutic effectiveness which such teaching is thought to engender but rather because I believe that a university should be devoted to the dissemination of accurate information about the subjects which it teaches. Moreover, since there is general agreement nowadays about the relevance of psychosocial (including economic) factors to human health and illness, it follows logically that such teaching belongs in medical schools. It is, however, a different matter to expect internists and general practitioners to be able, in addition to their present tasks, to do effective work in psychological diagnosis and treatment as well. This expectation, which is prevalent today, has many enthusiastic proponents. It is, of course, a good idea. But how much of this is feasible? This question is important, for if such a program is not feasible, what good is it to advocate it, except, once again, to make the advocates feel like "progressive humanitarians" (in contrast to presumably unfeeling "medical reactionaries"). What might be feasible in the future, of course, is often difficult to decide in advance. It is worth while to emphasize, however, as did Oppenheimer, that the world is becoming more, not less, complex, and that the combination of such skills as those of a competent medical physician on the one hand, and of a psychotherapeutic physician on the other, may prove to be impossible to unite in one person. Indeed, the evolution of medicine and psychiatry suggests that even within these two large domains, the multiplicity of techniques is such that more subdivisions will arise. The "ideal doctor" who knows all of medicine and can take care of the "whole patient" may, therefore, be more readily found in the past, I think, than in the future.

Who Is "Responsible" for the Patient?—Who, then, will take care of the patient and "assume responsibility," as we are wont to say, for him? I cannot answer this question. Indeed, I feel it would be presumptuous for anyone to arrogate to himself this decision. Such a question presupposes that the hypothetical "patient" occupies a position in relation to the physician not unlike the small child does in relation to his parent. Usually, this is not—or need not be—the case.[12] The person who must therefore assume responsibility for the patient is the patient

¶ I may note here briefly that there is a widespread, though probably not always fully conscious or overtly recognized, belief among physicians that they are "natural born" psychologists and psychotherapists. This explains why they so commonly advise patients about vital personal matters, such as marriage, divorce, or when to have or not have a child. It is a part of their social role and self-concept to know all about, and to be able to deal expertly with, "everyday human problems." We touch here on what Ernest Jones many years ago aptly described as the God complex.[4]

himself. This is not said to minimize the physician's "professional responsibility." It is, however, a part of this responsibility, as I see it, for the physician to make clear, both to himself and to his patient, what he can do, what he cannot do, and what others may be able to do for the patient. It follows, finally, that it is the physician's and the psychiatrist's role to assist the patient, whenever this is indicated or necessary, to recognize his own "responsibility" toward his life, in sickness as well as in health. Often the patient is prepared to "dump" this "responsibility" onto the physician, usually without realizing the dangerous consequences that may result from such action. It is for this reason that it is often easier for the physician to "accept this responsibility" (as we say), than it is to do anything else. Any other alternative immediately involves both patient and physician in an exchange of significant messages, an undertaking in which the physician may be blocked from the outset by his fear of losing his patient. Thus it happens that the physician will overtly accept "responsibility" for the patient (in some very large sense), while covertly he remains massively disengaged. There is an old French saying that is pertinent in this connection. It is *noblesse oblige*, which means that nobility is an obligation. So is "responsibility." Is it then not "irresponsibility" to accede to, and to take on, a "responsibility" greater than one can fulfill? I think it is likely that this passive attitude of physicians toward their responsibility to patients—and its proper limits—accounts in large part for the mounting dissatisfaction which patients feel about medical care, the technical excellence of which is at the same time undisputed.[2]

The Problem of "Differential Diagnosis."

Another practical consideration which follows from this line of thought relates to the so-called differential diagnosis between organic and psychological illness. The patient presenting himself with a mental symptom, such as a delusion of grandeur, may have paresis or paranoia; another suffering from fatigue and weight loss may have pernicious anemia or a depression. (Of course, this is not necessarily a matter of either-or, but I shall try to simplify the problem somewhat, to make my point.)

The social roles of physician and psychiatrist are important factors in determining whom the patient will consult first. In either case, in the large majority of instances, the so-called differential diagnosis is, or should be, a trivial task for the average unbiased examiner. Naturally, if the physician believes that every patient is "physically ill until proven otherwise," the establishment of a correct estimate of the problem at hand can become a colossal problem, perhaps in much the same way as it would be a great problem for most of us to prove our "innocence" merely because some members of the community are criminals. While I believe that in most cases this problem of differential diagnosis is a simple one, it is nevertheless true, and important, that in some instances it poses a very difficult task indeed. In such cases there can be no substitute for painstaking studies, if necessary, of both a physical and a psychological nature. Moreover, it must be remembered that just as it is sheer nonsense to speak of making a psychological diagnosis by "excluding organic illness first," so it is also a mistake to believe that the positive identification of a psychosocial disturbance has any bearing whatsoever on the possible presence or absence of bodily disorder. Physical methods of examination can establish only whether a physical disorder is demonstrable or not. Lack of such findings does not mean, as we know only too well, that a serious disorder may not exist. Lastly, as mentioned, psychological methods of examination have at most a remote, suggestive, bearing on whether or not the bodily machinery is disordered. Obviously, only physical methods can be used to "diagnose" bodily disease, and when such methods fail, we can take our pick between (1) believing that the patient is "healthy," (2) believing that he is "ill" and that in due course we

shall discover the nature of his disease, or (3) assuming that his symptoms are "psychogenic." The physician will communicate, of course, to the patient, in one way or another, what he thinks the problem is. Accordingly, in the first instance the patient will tend to be thought of as a "malingerer." [8] In the second, he will think he is suffering from an as yet undiscovered disease—probably very serious—and he may thus be started down the road of an iatrogenically stimulated hypochondriasis. And, in the third instance, the patient will be made to feel that he has no "organic disease" (which may, of course, be an error), that he is "neurotic," and that he should consult a psychiatrist. Clearly, all three alternatives are undesirable. There remains the possibility of the physician limiting himself to informing the patient about the absence of significant findings and the corresponding absence of any need, at this time, to take further medical action. Any further inferences would be then left up to the patient. I think this approach to the problem might be often useful (or least harmful) for the patient, but it is difficult for the doctor, for it puts him into a relatively more inactive position than that to which he is accustomed. Yet, by such "activity" the physician tends to keep his patient from taking "responsibility" for the management of his illness (and his "life," in a social sense).

The Changing Social Role of the Psychiatrist.—Around the turn of the last century, at about the time when Freud made his first discoveries, the psychiatrist's social role was either that of a physician in a hospital for the insane or that of a physician for diseases of the "nervous system." I think it was principally for this latter reason that Freud and his colleagues saw so many cases of "hysteria." [1] Patients with pains, paresthesias, and paralyses of all kinds naturally went—either spontaneously or by referral—to "neuropsychiatrists," physicians who specialized in those parts of their bodies which were thought to be affected. Today, patients who have complaints referable to their bodies still go to physicians (including neurologists), not to psychiatrists. The psychiatrist's role today is that of a medical specialist who treats only those who are healthy in body! (Think of the cartoon, for example, in which the patient is shown in his doctor's office, after a physical examination, exclaiming, "Thank God, it's all in my body!") This change in social roles has not been given as much attention as I think it deserves. Two relevant considerations will be mentioned in this connection. The first is that some patients go to the psychiatrist much more readily today than they did in Freud's day. These are the people who are troubled in various ways by their own behavior or personal relationships and who know that psychiatrists are professional men capable, sometimes, of helping with problems of this sort. On the other hand, the myriad of patients with disturbances in bodily feelings (or with functional paralyses simulating neurological disease), who were so commonplace in Freud's time, are relatively rarely seen by psychiatrists nowadays. The reason for this is that as long as a person complains about his (or her) body, it is entirely proper for him to consult a physician. These patients are therefore seen by general practitioners, internists, neurologists, neurosurgeons, and all the other medical and surgical specialists as well. They are given examinations, tests, and often various forms of treatments, the justification for which is based either on some slight deviations in one of the numerous tests or on the medically accepted practice which provides a routinized "schedule of treatments" for specific symptoms (e. g., diathermy and analgesics for pain, antacids and antispasmodics for abdominal distress, vitamins for fatigue, etc.). Those in whom absolutely nothing can be found that can be treated by physicochemical means are either "reassured" or castigated and/or sent away. Some "shop around." Few are sent to psychiatrists, and still fewer go to them and remain for treatment. The point that I am making is that patients with disordered bodily feelings

(usually) expect physical, not psychological treatment. After all, they are the products of the same culture that has moulded the physician.

Bodily Symptoms as the "Language" of Current Medical Practice.—This similarity between the patient's and the physician's attitudes toward complaints ("symptoms") referable to the body touches on the second consideration to which I wish to draw attention. I refer to the need to translate and to express certain experiences—particularly those relating to intimate personal relationships and feelings—into other than ordinary verbal language. This need arises, of course, as a result of cultural and social influences. However variable these influences may be in Western cultures, they all necessitate the avoidance of the open expression of various personal feelings. Freud[1] was naturally first struck by the avoidance of sexual and money matters in his social milieu. Viewed in terms of "language" and "social roles," what happens in many medical situations may be briefly described as follows. The patient knows (that is, has learned) that he is not supposed to complain about, say, sexual and marital problems. These things, and many others, are not supposed to be talked about. In even very intimate marital or friendly relationships the discussion of such matters is not usually condoned. If there is trouble in these sectors of one's life, something, of course, has to give. The psychosocial pressures then become "translated," so to speak, into other idioms, and find expression in derivative forms. Some literary creations are one such idiom. Bodily symptoms are another, much commoner, outlet.[10] Thus, while the patient may feel unable to complain about personal unhappiness, he can complain about dizziness, palpitation, and pain or itching in this or that body area. The physician, similarly, feels disinclined, and unprepared, to function as an aid to the patient in his personal troubles; but he does feel it to be his proper function to communicate with him in the language of his bodily feelings, that is, in terms of "possible bodily disease." The prohibition of certain highly "personal" topics as subjects of "conversation" also finds expression in the traditional medical use of Latin words (which is gradually diminishing), and in countries, or areas, where there is a strong Roman Catholic influence, in the explicit barriers which are put between patient and physician in relation to matters of procreation.

The Psychiatrist as "Translator."—Viewed in this light, the psychiatrist's role is, among others, that of a translator and interpreter of a foreign language. He must translate the patient's body language (e. g., "I have pains in my chest") into the language of everyday speech (e. g., "I am unhappy with my wife, job, religion, etc."). It is important to note, in this connection, that when patients go to physicians, they do not expect—and as a rule, do not welcome—such efforts at translation. They wish to speak in the idiom in which they address the physician, namely, in the idiom of bodily disorder. Moreover, it is clear from the model that the problem is not simply one of translation. The problem is perhaps better formulated by noting two important facets of it. First, there is the matter of recognizing that there is a problem of translation at hand, and second, there is the question of what to do about it. This is a problem that obviously involves both the physician and the patient, and hence it cannot be decided by the physician alone. While physicians are accustomed to deciding what patients ought to do, this procedure cannot be followed in the sort of situation under consideration. Instead, the physician should engage the patient in an attempt at exploring whether the latter can address himself to his problem in some idiom other than that of bodily feelings. Translation, therefore, is not an immediate therapeutic goal, but is merely a necessary prerequisite for tackling the problem in another idiom, in which, perhaps, it is more readily discussable and soluble.[10] Moreover, to tell the patient only that translation is necessary ("Your troubles are probably psychogenic,

therefore, you should go to a psychiatrist") is also of no service to him, unless two basic conditions can be fulfilled. First, both patient and physician must be able to converse in the new idiom (ordinary language, in this case) about the problem at hand, and second, there must be the likelihood that the original problem, rendered anew in translated form, will be more readily soluble than it had been before. Unless both of these conditions are fulfilled, persistent communication between patient and physician, in the idiom of bodily feelings, may be the best (least harmful) solution for everyone concerned.

On Referring the Patient to the Psychiatrist.—It is always possible, however, that the patient may be interested in, and quite capable of learning, another language. Or, he may already be in possession of another language but may be afraid to say so lest he offend his (apparently) less polyglot physician. For these reasons, it would be good medical practice—or so I would like to suggest—if physicians could learn to relinquish, when necessary, the language of bodily disease and would introduce, at appropriate times, the topic of "translation." By this I mean, concretely, informing the patient that, in his opinion, further inquiries along physical ("medical") lines are not indicated at this time. The physician could then add that the patient has one of three choices open to him. These are as follows: 1. If he strongly feels that a bodily disorder is the cause of his troubles and that it was simply not discovered (this possibility should, in all such cases, be openly acknowledged), he should feel free to consult another physician. 2. He may choose to do nothing and wait to see how things develop. Reexamination at a later date may help to reveal a bodily disorder that had formerly eluded detection. 3. He may wish to explore the possibility of resolving his troubles by psychological means, that is, by thinking about his life and human relationships. He (the patient) may do this, moreover, alone, with someone close to him (e. g., wife, husband, friend, or medical physician if the latter is so inclined), or, finally, with the aid of a psychiatrist. Only the patient's response to such medical advice can determine the appropriate course for him. Also, if the patient wishes to know, his physician should be forthright in telling him which of these three alternatives he favors. Then, if the patient does decide to consult a psychiatrist, the possibilities of more extensive translation, and of "working through" in a new idiom, can be further explored.

Summary

It was my purpose in this essay to discuss the theories and practices of medicine and psychiatry in terms of their characteristic scientific methods and social roles. The common-sense view which regards psychiatry as a branch of medicine dealing with the "mind," while not quite devoid of some social usefulness at present, is scientifically worthless and is often a source of confusion in medical practice and, particularly, in the professional relationship between physicians and psychiatrists.

The characteristic techniques of medicine are based on physics and chemistry—those of psychiatry, on psychology, sociology, and philosophy. The object of study in medicine is the human body as a machine; in psychiatry, the person as a social being. Neither can rightfully lay claim to self-aggrandizements, be these clothed in "scientism," holism," or "humanism."

The social roles of physicians and psychiatrists vary markedly from time to time and from place to place. At present, in the U. S. A., the physician is looked upon as primarily an expert "tester" and "repairman" of the human body. Only lip-service is paid—both within the medical profession and outside of it—to the physician as a personal advisor for people in trouble. The latter role has been assigned to, and has been accepted by, psychiatrists. They are regarded as "psychotherapists" who take care of only those who are healthy in body.

Several practical applications of this line of thought are noted. In regard to medical education it is suggested that the problem

of how much psychiatry ought to be taught in medical school is more complicated than it would appear from the usual arguments (which focus on content) about this topic. The present tendency is to "compensate" for the heavy "organic" emphasis in past medical education by loading the curriculum, and the student, with "more psychiatry." Today education in all fields faces the problem of needing to encompass increasing amounts of "material" within essentially inelastic curricula. This problem cannot be met by expanding the duration of formal education. It is better met by developing adequate systematizations and the teaching of principles rather than that of "data" and techniques. Thus, depending upon the future social role of the physician, it is conceivable that the teaching of either much less—or perhaps of much more—psychiatry might be appropriate for the undergraduate medical curriculum.

Concerning the other practical implications of this thesis, brief comments are made about the following problems and topics: the problem of "responsibility" for the patient, the "differential diagnosis" between organic and psychological illness, the changing social role of the psychiatrist, bodily symptoms as "language" and the psychiatrist as "translator," and, finally, referring the patient to the psychiatrist.

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Pulmonary Actinomycosis

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Infections in man by Actinomyces bovis usually have involved the cervicofacial, abdominal, or thoracic organs. The first two of these occur more frequently, pulmonary actinomycosis being noted in only about 15% of all clinically reported cases. The fungus is an endogenous organism of the mouth and gastrointestinal tract. Therefore, it may be a cause of disease in any portion of the United States or the world. It is more often seen, however, in certain rural sectors where either the external environment may be more frequently contaminated by the fungus or more likely where oral hygiene is poor. The need for recognition of all forms of the infection has taken on additional interest, since pulmonary involvement may be mistaken for carcinoma of the lung and because current therapies with various antibiotics, although frequently of prolonged duration, are now curative. It was thought, therefore, worth while to review the roentgenographic findings of pulmonary actinomycosis and to report typical cases that we have encountered in recent years.

Turner's [1] excellent description of actinomycosis of the lungs, in 1926, divided the pulmonary forms into essentially two types. The first, or early, form begins with involvement of the hilum extending into the lung, along the bronchi, causing fibrosis in these areas. Secondary parenchymatous involvement, with or without abscess formation, later may extend to the pleura and, subsequently, may involve the pleural cavity itself or the ribs. Decker's [2] and Kay's [3] descriptions are very similar. In the days before effective antibiotic therapy the early stage usually proceeded into the later stage, with involvement of the pleural cavity or ribs, producing a characteristic and diagnostic x-ray picture of actinomycotic infection. Peabody,[4] in a recent review, describes varying sized areas of consolidative pneumonia, often with small round lucencies which make it indistinguishable from pulmonary tuberculosis. Dense parahilar lesions simulating bronchial neoplasm were considered by him to be most unusual.

Today the patient is apt to have some form of chemotherapy, which arrests the infection before it reaches pleura or ribs, and he arrives at the hospital with a picture of disease limited to the lung. During the past three years we have encountered three cases of proven pulmonary actinomycosis. Two of these were admitted to the hospital with a presumptive diagnosis of carcinoma of the lung. In none had there been extension to the pleural spaces or the surrounding bone. In retrospect, it appeared that there were certain fundamental similarities in the x-ray picture which although previously described were worth reporting, since the findings do not currently seem to be well recognized.

Report of Cases

CASE 1.—A 59-year-old white male cook was admitted to the hospital on Dec. 11, 1952, complaining of productive morning cough with occasional hemoptysis of approximately two and one-half months' duration. A 10 lb. weight loss occurred, and immediately prior to admission some

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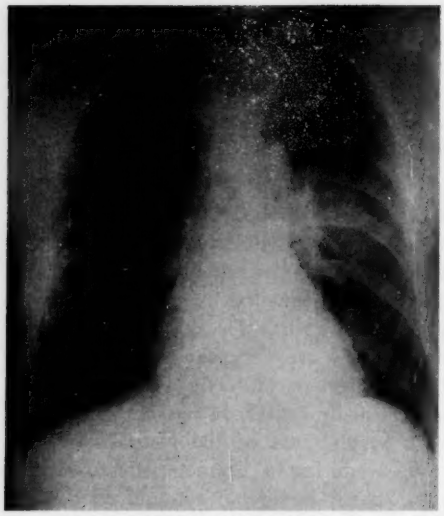


Fig. 1 (Case 1).—Chest x-ray, Dec. 19, 1952. There is an area of diffuse fan-shaped consolidation posterior to the left hilum in the apical segment of the left lower lobe. From this area increased lung markings radiate toward the base. The right lung is clear.

hoarseness had appeared. He gave a history of pneumonia six years before and had smoked more than one pack of cigarettes a day for many years. There were no other symptoms except those related to the lungs.

Physical examination revealed a well-developed man. Temperature was 99.6 F; the chest was clear to examination; there was a Grade 2 apical systolic murmur, without cardiomegaly. Findings on laboratory examination were as follows: the urine concentrated to 1.022 without albumin; WBC, 7450, with a normal differential count; hemoglobin value, 13 gm. Sputum was cultured for pathogens and acid-fast organisms, but not for fungi, and all were negative. Chest x-ray Dec. 19, 1952 (Fig. 1) revealed an area of diffuse fan-shaped consolidation posterior to the left hilum in the apical segment of the left lower lobe. From this area increased lung markings radiated toward the base. The heart was not enlarged. Bronchoscopy was performed, and an essentially normal bronchoscopic examination resulted. After a three-week period of observation without alteration in the size of the pulmonary lesion, it was felt that the possibility of a carcinoma of the lung was most likely and thoracotomy was recommended and accepted. This was performed, and a firm mass with marked adhesions to the pleura posteriorly was found, which involved the upper portion of the left lower lobe. Upon complete freeing of the lung, it was determined that the entire lesion was confined to the left lower lobe, and a left lower lobectomy was completed without incident. The cut surface of the lung presented a grayish-yellow granular appearance which was firm and nodular, with some intervening crepitant softer areas. Microscopically, there was marked fibrosis, both interstitial and alveolar, with organization of previous alveolar exudate and areas of acute cellular infiltrations of lymphocytes and plasma cells. A number of abscesses, 3 to 4 mm. in diameter, filled with polymorphonuclear leukocytes, were present, in the center of which were typical Actinomyces organisms with a peripheral acidophilic fringe of radial club-shaped processes. The microscopic diagnosis was chronic p. umonitis due to actinomycosis. Unfortunately, no cultures were made of the resected lung tissue.

Postoperatively, the patient did well except for slow reexpansion of the left upper lobe. On receipt of the diagnosis from the pathological department, he was given penicillin, 1,600,000 units per day, for one month. With this therapy, there was slow but steady clearing of the atelectatic areas in the upper lobe without further evidence of significant pulmonary infection. The patient was discharged six weeks after the lobectomy and has been seen repeatedly during the succeeding two and one-half years. There has been no evidence of recurrence of his pulmonary infection.

Case 2.—A 46-year-old baker was admitted April 27, 1953, complaining of progressive cigarette cough of 1 year's duration, 3 months of severe anorexia and weakness, and 10 days of left anterior pleuritic chest pain associated with fever and chilliness. There had been some yellow sputum, night sweats, and mild hemoptysis. He had received three injections of penicillin during the week prior to admission and on the day of admission had developed a papular rash, with pains in knees, ankles, and elbows. A 25 lb. weight loss had been experienced during the year of his present illness. He had smoked one to two packages of cigarettes a day for 25 years.

On physical examination, the temperature was 100.2 F, pulse, 100. He was a thin middle-aged man in moderate distress and appearing chronically ill. There was dullness in the lower two-thirds of the left chest, with bronchial breathing and showers of inspiratory rales over that area. A diffuse papular rash covered all areas of the skin; shotty lymph nodes were present in all regions, and there was tenderness of many joints. The white cell count was 11,000, with 84% polymorphonuclear leukocytes; the urinalysis, normal; hemoglobin, 11 gm. Sputum cultures revealed only α-Streptococci and catarrhalis. Repeated examinations were negative for acid-fast bacilli.

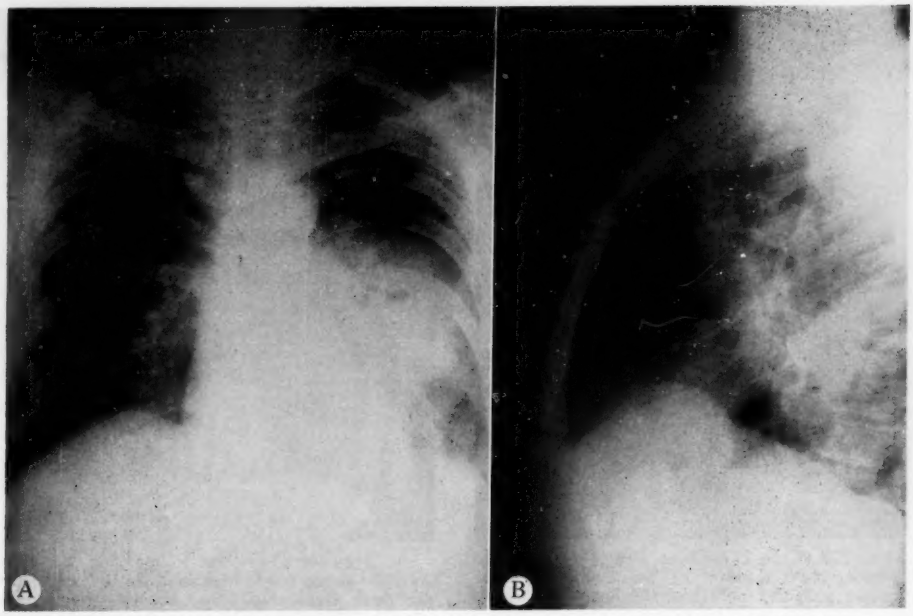
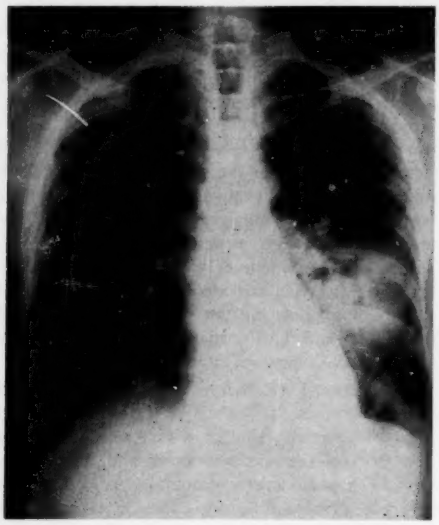


Fig. 2 (Case 2).—Chest x-ray, April 28, 1953. There is extensive diffuse fan-shaped consolidation throughout the left lower lobe, radiating from the left hilum posteriorly and to the base. Small pleural effusion is present, blunting the left costophrenic angle. On the right there is similar diffuse, less extensive consolidation in the anterior segment of right upper lobe. In both consolidations, there are suggestive areas of rarefaction.

Fig. 2C (Case 2).—Chest x-ray, May 1, 1953. Overexposed bucky film. Findings essentially unchanged. Areas of rarefaction stand out with better contrast. They are consistent with cavitations.



X-rays of the chest taken April 28, 1953 (Fig. 2*A* and *B*) revealed an extensive diffuse, fan-shaped consolidation throughout nearly the entire left lower lobe, radiating from the left hilum posteriorly and toward the base. A small pleural effusion blunted the left costophrenic angle. On the right there was a similar diffuse, less extensive consolidation in the anterior segment of right upper lobe. In both consolidations, suggestive areas of rarefaction were noted. An overexposed x-ray film taken May 1, 1953 (Fig. 2C) brought out the areas of rarefaction in better contrast and was consistent with multiple cavitations.

He ran a febrile course, with temperatures between 100 and 102 F, continued to lose weight, and appeared critically ill. Because of the similarity of the pulmonary process to Case 1, smears and cultures were made for Actinomyces. He was given chlortetracycline (Aureomycin), 0.5 gm. every six hours, on April 30, and this medication was continued in this dosage for the next five weeks. Subsequently, numerous cultures grew out A. bovis, which was highly sensitive to penicillin, streptomycin, chlortetracycline, and oxytetracycline (Terramycin). Because of a history of apparent sensitivity to penicillin, therapy with chlortetra-

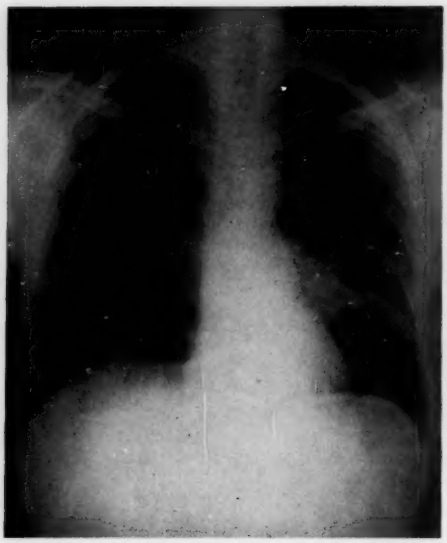


Fig. 2D (Case 2).—Chest x-ray, May 20, 1953. Considerable resolution of pneumonic consolidation on both the right and the left.

Fig. 3 (Case 3).—Chest x-ray, Aug. 23, 1955. Lung fields are emphysematous. In the left upper lobe there are several linear fibrotic strands, with adjacent thickened pleura and several minute calcifications. On the right the hilum is prominent and there is a well-defined mass or consolidation posterior to it measuring 3×4 cm. in size. Right horizontal fissure is slightly elevated, and several minimal fibrotic strands are seen in the right upper lobe. These findings did not change during a month of hospitalization.

cycline was continued, 2 gm. daily. Further x-rays on May 20, 1953 (Fig. 2D), three weeks after onset of therapy, revealed considerable resolution of the process of both the right upper and left lung fields. Cultures of his sputum after initiation of therapy were repeatedly positive for Actinomyces for two weeks but were negative thereafter. Although the lungs had not entirely cleared at the time of cessation of therapy, the improvement continued, and a normal chest x-ray was obtained two months after onset of therapy. Follow-up x-rays 18 months later were normal.

Case 3.—A 66-year-old former schoolteacher was referred to the hospital on July 19, 1955, with the diagnosis of tumor of the right hilum. He had been hospitalized for three years, between 1919 and 1922, for recurrent attacks of pleurisy and pulmonary tuberculosis. He smoked from one to three packages of cigarettes a day during the interim years and experienced moderate cough. One month prior to admission he had noted an increase in the cough without constitutional symptoms but accompanied by pleuritic pains in the right peristernal area. Because the chest x-ray that was taken showed changes other than those which had been noted a year before, he was admitted for observation.

On physical examination he was seen to be an undernourished man. Except for minimal emphysema, physical examination was not remarkable. Laboratory urinalysis was normal, as were blood cell counts. Serology was negative. Repeated sputums and gastric aspirations were negative for acid-fast bacilli and other bacterial organisms. A single fungus culture was negative. Chest x-ray on Aug. 23, 1955 (Fig. 3) revealed emphysematous lung fields. In the left upper lobe there were several linear fibrotic strands with adjacent thickened pleura and several minute calcifications. On the right the hilum was prominent and a well-defined mass or consolidation posterior to it, measuring 3×4 cm. in size, projected from it. The right horizontal fissure was slightly elevated, and several minimal fibrotic strands were seen in the right upper lobe. These findings did not change during a month of hospitalization. Laminography confirmed the impression of a mass posterior to the right hilus, with hilar lymph node enlargement. Bronchoscopy and a supraclavicular node dissection were normal. A barium swallow did not reveal any other lymph node involvement. Papanicolaou washings of the bronchi were negative for tumor cells.

The patient was observed over a period of two months. Because of failure of the mass to change in a two-months period of observation, it was felt that thoracotomy was warranted, and, accordingly, a right lower lobectomy was performed. There was considerable difficulty exposing the lung be-

cause of dense adhesions throughout the lower lobe, through which it was possible to palpate a firm mass adjacent to the posterior parietal pleura which had the characteristics of carcinoma. The lobe was removed with the adjacent lymph nodes. On cut section a yellow-gray nodule about 2.5 cm. in diameter was noted, with margins that blended gradually into the surrounding parenchyma. Microscopically this nodule consisted of lung tissue with an exudation into alveoli of inflammatory cells. There were small abscesses in the center of which were actinomycotic granules forming the nidus of the abscess. Postoperatively, the patient did well, and he was given a one-month course of penicillin, 1,500,000 units per day. At the conclusion of this he felt well and chest x-ray revealed no evidence of recurrence of the mycotic infection.

Comment

The central location of the lesion in each of the patients is of some interest and is in accord with the previous descriptions of early pulmonary actinomycosis. The patients were of the age group in which carcinoma of the lung is a considerable concern, and they all had long-standing histories of cigarette smoking. Adequate fungus studies were made in only one patient, but the diagnosis probably should have been established in the other patients without surgery had this been attempted prior to operation.

The roentgenological findings in three cases of pulmonary actinomycosis present a certain common denominator which in our opinion is significant enough to enter into the differential diagnosis of pulmonary lesions. The finding is an area of fan-shaped consolidation near hilum or radiating from it, usually posterior, frequently involving apical segment of a lower lobe. It may not be easily differentiated from the usual appearance of neoplasm or pneumonia. In the absence of definitive bacteriological findings, differential diagnosis of pulmonary actinomycosis should be considered if above-described roentgenological findings are present.

Summary and Conclusions

Three cases are presented of actinomycosis without significant pleural or thoracic wall involvement, proven by culture and operation. Carcinoma of the lung was erroneously diagnosed in two patients. An x-ray picture of similar nature was present in the three patients, there being a characteristic origin of the lesion at the hilus, with a fan-shaped or lobulated consolidation extending into the parenchyma of the lung, particularly along the bronchi. Secondary lesions were occasionally present in other portions of the lung.

It is recommended that patients in whom carcinoma is suspected, with central hilar involvement radiating into the periphery of the lung and with suggestive lymph node involvement, have a careful smear and culture of their sputum for actinomycosis done prior to surgery.

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Studies of the Bone Marrow in Immunological Granulocytopenia

Following Administration of Salicylazosulfapyridine

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With the Technical Assistance of Mary Tibbetts, B.A., M.T.

Severe granulocytopenia associated with a decreased resistance to infections was recognized as a new disease syndrome in 1922.[1] Nine years later Kracke directed attention to the causal relationship of drugs.[2,3] Shortly thereafter, Madison and Squier [4] collected evidence that, of all drugs then in use, aminopyrine was most clearly implicated. These authors were also the first to reproduce granulocytopenia by the administration of aminopyrine to patients who had recovered from previous episodes associated with the use of an aminopyrine barbiturate preparation. A dose of 0.3 gm. by mouth was followed within hours by fever, malaise, and a precipitous fall in granulocytes in one patient. The second exhibited only a sharp drop in granulocyte count following the medication. These clinical experiments were repeated and confirmed by others.[5,6]

Plum's [7] studies of the bone marrow during the administration of aminopyrine to three sensitive patients are of particular interest. He noted a decrease in the number of granulocyte precursors in the bone marrow smears preceding the fall of the peripheral blood cell count. During recovery an increase in granulocyte precursors preceded the reappearance of granulocytes in the peripheral blood. These observations appear to be in conflict with the prevalent concept of maturation arrest of a cellular marrow at the myelocyte or metamyelocyte stage as the mechanism of granulocytopenia. As pointed out by Dameshek,[8] in his monograph, the serial studies of Plum take precedence over single observations made after the disorder had developed and suggest that patients who exhibit a hyperplastic marrow are in a phase just preceding recovery. Death due to infection frequently prevented recovery.

When the sulfonamide drugs were introduced as antibacterial agents, severe granulocytopenia was the most serious complication of their administration. According to one survey the incidence was somewhat higher with sulfapyridine than with other derivatives, being 0.6% for granulocytopenia, including 0.3% who showed an absence of granulocytes in the peripheral blood.[9] Several features suggested that the severe granulocytopenia resulting from administration of sulfonamides was due to a mechanism similar or perhaps identical to that producing granulocytopenia following aminopyrine ingestion, which had been studied during the preceding years. The relatively low incidence with both drugs after variable periods of exposure indicated an altered reaction on the part of the individual characteristic of an immune response. The frequency with which fever, skin rash, and malaise preceded or accompanied the development of granulocytopenia was also consistent with the view that the reaction was a form of "antigen sickness."

Understanding of the pathogenesis of granulocytopenia in drug hypersensitivity has been extended by the recent studies of

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Moeschlin and Wagner.[10] By transfusion experiments these authors demonstrated the appearance of a granulocytopenic factor in the plasma after ingestion of aminopyrine by sensitive persons. An agglutinin for leukocytes was also found in the serum during the granulocytopenic phase. In a subsequent report [11] a patient with marked granulocytopenia after sulfapyridine medication was observed to have leukocyte agglutinins in the serum for several days after ingestion of that drug. As a result of their experience, Moeschlin and Wagner [10] postulated that the rapid disappearance of leukocytes of the peripheral blood, which occurred in their patients, depleted and exhausted the marrow. Plum,[7] on the other hand, postulated that marrow failure was the primary factor in the disorder.

The occurrence of severe granulocytopenia in a young man who had been receiving salicylazosulfapyridine for ulcerative colitis posed questions as to the complicity of the drug and its mechanism of action. The studies reported here were carried out in an attempt to answer these questions. The drug is an acid azo compound of salicylic acid and sulfapyridine (Azulfidine, Azopyrine, Salazopyrine), which has been widely used in the treatment of ulcerative colitis for more than 15 years. The infrequency of serious toxic effects, despite its chemical precursors, may be related to the low solubility and limited absorption. Mild depression of the leukocyte count has been observed frequently, but in only two patients has severe granulocytopenia been attributed to the drug.[12,13]

Report of Case

The patient was a 35-year-old white man who developed acute diarrhea during February, 1956, which failed to subside. During the second week blood and mucous appeared in the stools. He consulted a physician, and after stool examination, proctoscopy, and barium enema he was informed that he had ulcerative colitis. He was given salicylazosulfapyridine, 4 gm. daily in divided doses, and an antispasmotic, J. B. 340, (N-methyl-3-piperidyldephenyl glyconate methobromide) daily. The number of stools per day was reduced, and they became more formed. After five weeks of the medications he noted the appearance of a rash on the arms and hands that remained for three days. Concomitantly, he experienced chilly sensations and became feverish, with temperatures as high as 104 F. He also noted generalized headache, malaise, and anorexia. During the next two weeks his symptoms continued, the fever persisted, and he lost 15 lb. In spite of this he did not report to his physician and continued to take his medications. There was no change in bowel symptoms, which continued to be mild. Throat discomfort began several days before entry into the hospital.

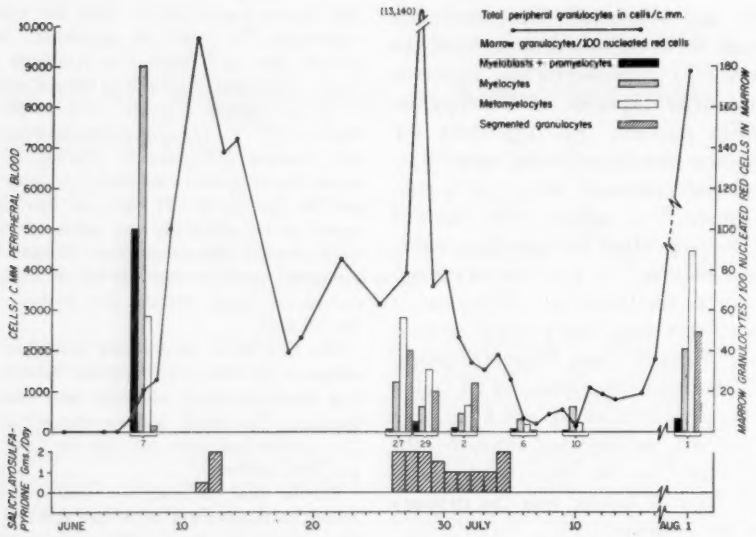
He had been hospitalized elsewhere on two occasions in 1953 for "nervous breakdown" and had received both electric- and insulin-shock therapy. The family history was not of interest. The patient had been married for five years and had two children.

At the time of entry he was acutely ill and rather withdrawn but in a fair state of nutrition. The skin was clear. The gum margins were inflamed, and the pharyngeal mucosa was hyperemic but not ulcerated. Lymph nodes were palpable in the anterior cervical chain and were slightly tender. There was no other significant lymphadenopathy. The chest was resonant throughout, and breath sounds were normal. The heart was not remarkable and blood pressure was 130 systolic and 80 diastolic. The liver and spleen were not palpable, and there was no abdominal tenderness or organ enlargement. The extremities were normal.

Granulocytes were not found in the blood smears. The initial leukocyte count was 1150, of which 1% were monocytes and 99%, lymphocytes. The platelets and red cells appeared normal in the blood smear. The hemoglobin was 12 gm/100 cc., and hematocrit, 36. Reticulocytes were 0.4%. The direct antiglobulin serum test was negative.

Urinalysis was not remarkable. Cultures of the throat and of the stool grew only normal bacterial flora. Chest x-ray was normal.

Course.—During the first 48 hours the rectal temperature varied from 103 to 104 F. On the second day the gums appeared more inflamed. Penicillin therapy, 600,000 units twice daily, was begun. On the third hospital day his temperature began to subside rapidly, and it was normal on the fourth. On the fifth day the total leukocyte count was 1950 and granulocytes were still not found in the peripheral blood smears. The following day the total count was 1900, of which 19% were granulocytes. Sternal marrow aspiration smears on the seventh day showed a large number of cells at the promyelocyte and myelocyte stage and moderate numbers of metamyelocytes. Segmented granulocytes were present but reduced in



The course of granulocytopenia caused by salicylazosulfapyridine. Recovery from the granulocytopenia found on admission to the hospital was followed by recurrence during readministration of the drug. Stages of granulocyte development found in seven marrow aspirations are expressed as the number per 100 nucleated red cells.

TABLE 1.—*Peripheral Blood Cell Counts* *

Date	Cells/Cu. Mm.		Differential Counts, %							
	White Cells	Granulocytes	Neutrophils		Myelocytes	Eosinophils	Basophils	Lymphocytes	Monocytes	Unclassified
			Segmented	Juveniles						
June, 1956										
1	1,150	0						99	1	
4	750	0						89	11	
5	1,950	0						84	14	2
6	1,900	361	6	13				68	10	3
7	6,000	1,020	16	1				45	38	
8	4,400	1,230	25	3				46	24	2
9	7,800	4,992	30	32			2	32	2	2
11	13,500	9,720	51	19			2	20	7	1
13	10,900	6,976	46	15	2		1	34	1	1
14	9,400	7,240	69	4	4			18	4	1
15	10,800									
18	5,100	1,990	39					55	6	
19	5,500	2,310	41	1				52	6	
22	9,600	4,320	45					50	5	
25	6,500	3,185	48	1				45	6	
27	7,000	3,920	56					40	4	
28	6,800	5,710	82	2				16		
28	14,600	13,140	90					7	3	
29	4,500	3,600	75	4			1	15	5	
30	7,300	3,940	53	1				41	5	
July, 1956										
1	4,300	2,365	55					42	3	
2	3,400	1,700	45	4			1	46	4	
3	3,300	1,518	44	1			1	51	3	
4	3,300	1,848	55				1	39	5	
5	3,300	1,353	29	9	3			56	3	
6	3,000	360	11	1				73	15	
7	4,300	258	4	2				85	9	
8	2,200									
8	3,100	434	2	12				81	5	
9	2,800	504	1	17				76	8	
10	5,200	104	0	2				6		
11	4,400	1,100	2	23				67	8	
12	5,300	954	3	15				77	5	
13	4,400	792	13	5				76	6	
14	4,000									
15	4,200	924	19	3				71	7	
16	4,400	1,760	35	5				54	6	
Aug., 1956										
1	12,100	8,833	73					25	2	

* Total leukocyte and differential counts during the course of granulocytopenia in a patient exhibiting signs of sensitivity to salicylazosulfapyridine; 400 cells were included in each count. The relationship of the differential counts to the patients recovery and readministration of the drug is shown in the Figure. The number of granulocytes per cubic millimeter has been computed.

number. Eosinophils were not increased. Red cell precursors and megakaryocytes appeared normal.

The hematological observations made during the course of his hospitalization are summarized in the Figure and in Table 1.

On the 12th hospital day he received 0.5 gm. of salicylazosulfapyridine by mouth. Frequent leukocyte and differential counts during the next 24 hours did not show a significant change. Salicylazosulfapyridine, 2.0 gm., was then administered without producing significant change during the next three days. Five days after the second dose the total leukocyte count was 5100 and the granulocytes, 1990 per cubic millimeter. During the subsequent three-day period there was a return of the granulocytes to normal levels.

After 14 days of observation, during which there was no response to a trial of J. B. 340, salicylazosulfapyridine therapy was again begun, with an oral dose of 0.5 gm. every six hours. Following the fourth oral dose he experienced chilly feelings and malaise and his oral temperature rose from a normal range to 101 F. At this time there was an abrupt rise in leukocyte count to 14,600 and a granulocytosis of 90%. A transient morbiliform rash appeared, but physical examination was otherwise normal. The medication was continued without recurrence of subjective symptoms. Leukocyte and total granulocyte counts returned to premedication levels.

On the fourth day the dosage was reduced to 0.5 gm. every 12 hours because of some reduction in granulocytes. On this regimen his total granulocytes did not appear to be further reduced during a subsequent four-day period. The dosage was again increased to 0.5 gm. every six hours. The following day the granulocyte count was 360 and the medication was discontinued. The granulocyte count remained low during the next 10 days, though the granulocytes did not disappear entirely from the peripheral blood. There was then a return of granulocytes and total leukocytes to a normal level.

During the periods of granulocytopenia the cells of that series developed basophilic granules within the cytoplasm. Lymphocytes and monocytes did not exhibit recognizable alterations in morphology. The platelet and reticulocyte counts and the hematocrit determinations remained within normal range throughout the course, as did erythrocyte morphology.

Bone Marrow Studies.—The patient's bone marrow was examined by sternal puncture aspiration seven times during this study. In each instance a differential count of 1000 nucleated marrow cells in consecutive microscopic fields was recorded. In addition the mitotic figures seen during the differential count were noted and classified as to red cell and granulocytic series.

Data obtained from the differential counts are recorded in Table 2 and are shown graphically in the Figure. Granulocytes have been equated to nucleated red blood cells in an attempt to develop a standard reference for comparison of the marrows. The first marrow was taken on June 7, early in the period of rapid return of the peripheral blood to normal. This specimen contained a marked relative and absolute increase in the immature granulocytes, compared with two marrows aspirated on June 27 and Aug. 1, at periods when the peripheral blood cell counts were normal and the marrow was presumably in a relative steady state. The second marrow was obtained just prior to readministration of the drug, on June 27. Two days

TABLE 2.—*Serial Bone Marrow Cell Counts* *

	June 7	June 27	June 29	July 2	July 6	July 10	Aug. 1
Differential counts, %							
Myeloblasts	1.4	0.3	0.3	0.4	0.1	0	0.2
Promyelocytes	20.1	0.5	2.	0.9	1.5	0.7	1.1
Myelocytes	39.2	9.6	9.1	5.4	6.7	10.8	13.8
Metamyelocytes	12.5	22.5	17.5	7.9	2.1	3.3	30.
Segmented granulocytes	0.7	15.8	11.3	13.8	0.6	0	16.5
Pronormoblasts	1.8	1.4	2.7	3.7	4.1	2.7	1.7
Normoblasts	19.8	38.6	53.1	54.3	74.2	64.7	32.3
Lymphocytes	4.4	11.	3.	13.1	10.2	17.4	4.2
Monocytes	0	0	0	0	0	0.1	0
Plasma cells	0	0.2	0.6	0.3	0.4	0.2	0.2
Megakaryocytes	0.1	0.1	0.4	0.2	0.1	0.1	0
Granulocytes/100 nucleated red cells							
Myeloblasts and promyelocytes	100.	2.	4.1	2.2	2.0	1.0	3.8
Myelocytes	181.	24.	16.	9.3	8.6	16.	41.
Metamyelocytes	58.	56.	31.	13.6	2.7	4.9	88.
Segmented granulocytes	3.2	40.	20.	24.	0.8	0	49.
Granulocyte mitoses/1,000 nucleated marrow cells	7.	2.	1.	1.	1.	1.	1.

* Differential counts of aspiration bone marrow smears; 1,000 nucleated cells were counted in each instance. Additional counts of 300 cells were substantially the same. From these data the number of granulocytes per 100 nucleated red cells was computed. Mitosis of granulocytic cells observed in the differential count was also observed and recorded. As shown in the Figure the marrow specimens were obtained during recovery from granulocytopenia following salicylazosulfapyridine therapy and during and following the readministration of the drug.

later there was a moderate decrease in segmented granulocytes, metamyelocytes, and perhaps in myelocytes, but the peripheral blood had returned to previous levels following the brief leukocytosis. On July 2, when the total peripheral granulocyte count had fallen to 1700, the marrow biopsy showed a further reduction in granulocytes, most marked in the myelocyte and metamyelocyte stages. During the subsequent four days the peripheral granulocytopenia continued. On July 6 the total peripheral granulocyte count had dropped to 360 and the drug was discontinued. The marrow at this time showed a reduction in all granulocytes, including myeloblasts and promyelocytes. Four days later, on July 10, the granulocytopenia persisted with a total peripheral granulocyte count of 104. The marrow at this time showed an absence of segmented granulocytes, but the number of metamyelocytes and myelocytes had increased slightly. The seventh marrow biopsy, taken Aug. 1, when the total peripheral granulocyte count and leukocyte count had returned to normal, exhibited only a moderately elevated metamyelocyte count.

The number of granulocyte mitoses per 1000 marrow cells is presented in Table 2. The dividing cells were normal in morphology and granulocytic, and erythrocytic precursors could be separated without difficulty. Seven granulocyte mitoses per 1000 nucleated cells were counted in the marrow taken at the beginning of the phase of rapid recovery from the first episode of granulocytopenia. Thereafter the number of mitoses was found to be 2 or 1 per 1000 nucleated cells in the marrows removed during the periods when the peripheral blood was normal or during the development and continuation of granulocytopenia. Scanning of entire coverslip preparations confirmed the data obtained by limited counts as to the number of mitotic figures in the marrow of June 7 as compared to any other period.

The cells of the erythroid series and the megakaryocytes were not detected to show changes in morphology in any of the marrow specimens.

Serological Studies.—The method of Dausett [14] was employed in the preparation of leukocyte suspensions and the observations of agglutination. Serum specimens were collected at regular intervals during control periods and during the time when medication was being given and granulocytopenia was present. The sera were stored at —20 C, and serological observations were carried out at one time. Both autologous and homologous Group O leukocyte suspensions were used.

All sera gave negative results, with the exception of that collected four hours after the initial dose of 0.5 gm. of medication on June 27, the first day of readministration of the drug, which produced consistently positive results with both autologous and homologous leukocytes. Serum specimens drawn during the control periods and following the development of marked granulocytopenia were consistently negative. Normal control sera also gave negative results. The addition of salicylazosulfapyridine, sulfapyridine, and sodium salicylate separately to serum aliquots and to samples of the cell suspension prior to the performance of the agglutination studies failed to produce positive results with any of the negative sera.

Skin tests were carried out in the manner described by Dameshek and Colmes.[6] The patient's serum was incubated with equal quantities of a saturated solution of salicylazosulfapyridine in 0.9% saline for four hours at 37 C prior to injection intradermally. This as well as the injection of the solution of salicylazosulfapyridine failed to produce a positive reaction, either immediate or delayed.

Comment

The suspected relationship of the granulocytopenia to the salicylazosulfapyridine medication was confirmed by its recurrence following readministration of the drug. The patient was not given sulfapyridine alone, so we do not know if the granulocytopenia was produced by the whole molecule or was dependent on separation of the drug into its component parts after ingestion. This study serves to reemphasize the fact that a sulfonamide derivative must be considered a potential cause of severe granulocytopenia. It also reminds us that fever, skin rash, or other symptoms developing in a person receiving any medication calls for reevaluation of the patient, particularly for hematological disorders including granulocytopenia. In a high percentage of instances of severe granulocytopenia related to drugs reported over the past three decades, systemic manifestations heralded the onset of an altered reaction to the medication. Patients receiving maintenance doses of drugs potentially dangerous should be told to discontinue the drugs and to seek medical attention if these symptoms appear.

It is not possible to be precise about the relative importance of increased destruction as opposed to decreased formation in the pathogenesis of the granulocytopenia, but the data permit some interpretation. The slow fall in granulocytes of the peripheral

blood and the concomitant decrease in marrow granulocytes could be explained by depressed formation through action of a toxin inhibiting cell metabolism and division. During the development of granulocytopenia, all stages of granulocyte precursors became less numerous in the marrow in comparison to nucleated red cells. The counts of the granulocyte mitoses indicate that there was failure to respond to granulocytopenia by an increase in the rate of cell division. The number of granulocyte mitoses per 1000 nucleated marrow cells remained within the same range as that found in the relatively normal marrows. The lag in proliferative activity after cessation of the drug may also be interpreted as additional evidence of marrow depression. The ability of the marrow to respond eventually to granulocytopenia was well demonstrated by the sample taken during the first recovery phase, when both granulocyte precursors and their mitotic activity were significantly increased. It is probable that a similar increase in marrow activity occurred during the second recovery period after the marrow count on July 10.

Increased peripheral destruction of granulocytes, on the other hand, would not explain adequately the granulocytopenia observed in our patient. A precipitous fall in granulocytes was not detected at any time. In addition, it is unlikely that peripheral destruction alone could have depleted the marrow without stimulating mitotic activity during the 10-day period of observation. Studies of leukopenia in dogs induced by leukophoresis indicate that the marrow responds to restore the peripheral blood to normal after the third or fourth day.[15] If increased destruction occurred in this patient, it is probable that it was of secondary importance compared to marrow depression in the pathogenesis of the granulocytopenia.

There are several reasons for believing that the granulocytopenia followed the development of an immune response to the drug in our patient. In common with others, he was able to take 4 gm. daily for five weeks without untoward symptoms. This suggests that he did not have unusual susceptibility to any inherent toxic properties of the drug. The initial reaction which probably preceded the granulocytopenia was characterized by fever, malaise, anorexia, and skin rash. A similar reaction followed by the onset of granulocytopenia occurred two days after the readministration of the drug in doses of 2 gm. daily. The rapidity of onset of the second reaction as well as its general pattern was consistent with an immune response. Finally, an agglutinin for leukocytes appeared in the patient's serum shortly after readministration of the drug. Leukocyte suspensions prepared from the patient's blood following recovery were agglutinated in this serum specimen to the same degree as suspensions of leukocytes prepared from normal controls. This autoactivity of the leukocyte antibody enhances its significance as a possible causative agent.

Koszewski and Hubbard [16] have reported studies of a patient observed to develop granulocytopenia following injections of mercurial diuretics. An agglutinin for normal leukocytes was present in the serum only during the first four hours after reinjection of the drug. In contrast to our observations, they were unable to demonstrate agglutination of the patient's own leukocytes. It is also of interest that transfusion of 300 cc. of whole blood withdrawn from their patient on the third day produced significant leukopenia in a normal recipient. This suggests that transfusion experiments may be more sensitive than the in vitro studies in detecting leukocyte antibodies.

If a leukocyte antibody was responsible for the granulocytopenia in our patient, why was it demonstrated in only one serum specimen? Several tentative explanations may be advanced. In various autoimmune diseases the antibodies are capable of combining with antigen receptors within the same organism. It follows that the amount of antibody formed and its avidity as well as the total adsorptive capacity of the receptors must be significant variables in de-

termining the presence or absence of free serum antibody. If the rate of production of the antibody is relatively slow, it may be adsorbed with sufficient rapidity to allow for little excess to remain free. It is possible that the appearance of a leukocyte agglutinin in the serum during the first period after administration of the drug was due to a relatively rapid formation at that time so that an excess accumulated in the serum in sufficient concentration to be detectable. It is also possible that lack of sensitivity of the methods used may have contributed to our inability to demonstrate the leukocyte agglutinin in some serum specimens. Little is known of the concentrations of antibody necessary to produce agglutination of leukocyte suspensions prepared with present techniques. The titer of leukocyte agglutinins observed in this laboratory have frequently been low. Positive sera have not produced agglutination when diluted, suggesting that the margin between demonstration of an agglutinin and failure to do so is narrow. In addition to the foregoing factors, the response of the marrow to administration of the drug suggests that the principal site of action of an antibody may have been the granulocyte precursors. If the marrow cells adsorbed the antibody with equal or greater avidity than granulocytes of the peripheral blood, this would also help to account for the low titer of free serum antibody in our patient.

This study raises many questions concerning the nature of the immune reaction to drugs and the influence of qualitative as well as quantitative variations of antibodies in relation to the clinical syndrome. In our opinion, the mechanism by which a particular drug activates an immune disorder to produce granulocytopenia has not been established. It appears from the data available that in some cases in which the response is almost immediate the protein representing the antibody remains in the circulating plasma of the sensitive person and is activated by the addition of the drug in vivo and sometimes in vitro.[11] The agglutination of leukocytes of normal persons without prior exposure to the drug suggests that the antigen involved is a normal constituent of human leukocytes. It may be that differences in the rapidity of onset of granulocytopenia depend largely on the quantity of antibody formed. An excess of antibody may be necessary to produce rapid removal of granulocytes from the peripheral blood, as observed by Moeschlin and Wagner.[10] If, on the other hand, the drug in question is not easily absorbed or is given in small doses, the antibody formed may be insufficient to produce rapid peripheral destruction and granulocytopenia is brought about by a slower process of bone marrow inhibition, as occurred in our patient. Presumably this latter effect might also be produced by a limitation in the antibody protein available in the person receiving the drug.

Summary and Conclusions

Severe granulocytopenia was encountered in a young man who had received prolonged medication with salicylazosulfapyridine in the treatment of ulcerative colitis. The relationship of this drug to the granulocytopenia was established by a recurrence during readministration of the drug.

An induced episode was characterized by a gradual reduction in peripheral blood granulocytes and a concomitant decrease in granulocyte precursors in the marrow. Mitotic activity of granulocyte precursors failed to increase during the 10 days of granulocytopenia. Decreased production rather than increased peripheral destruction is postulated to have been the principal mechanism in this patient.

A leukocyte agglutinin appeared in the patient's serum four hours after the initial dose of medication but was not demonstrable thereafter. It is suggested that an antibody activated by the ingestion of the drug may have acted chiefly on the marrow cells and depressed formation of granulocytes.

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Absence of a Relationship Between Ceruloplasmin and Nonspecific Hyaluronidase Inhibitor

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Several years ago Wattenberg and Glick noted that a purified nonspecific hyaluronidase inhibitor was blue in color and suggested that this pigment was ceruloplasmin.[1] Further fractionation increased the potency of the inhibition tenfold but not the color as measured spectrophotometrically. On the basis of this observation the authors concluded that the enzyme-inhibiting factor probably was not related to the copper-bearing protein. The identity of the inhibitor and a heparin-like polysaccharide was suggested.

Later, Newman and his associates obtained a highly purified hyaluronidase inhibitor by a combination of chemical procedures and zone electrophoresis.[2] No heparin was present, but the presence of glucosomine and uronic acid again suggested that the inhibitor might be polysaccharide. These investigators did not mention that their concentrated material had a blue color, nor did they report measurements of its copper content.

Kühnau[3] has stated that copper in complex binding has a profound antihyaluronidase effect not shared with ionized copper. A chart demonstrating such inhibition was presented, but the experimental details were not made available. He drew the conclusion that ceruloplasmin was a natural regulator of hyaluronidase activity and that the rise of plasma copper during infection was a natural defense against bacterial hyaluronidases.

These conflicting points of view prompted a study in which concentrations of serum copper, present principally as ceruloplasmin,[4] were compared with those of the nonspecific inhibitor of bovine testicular hyaluronidase in human sera. In addition, the antihyaluronidase effect of purified ceruloplasmin was investigated.

Materials and Methods

Sera were collected from healthy, pregnant, and sick human beings. Copper-free needles, syringes, and glassware were used. Methods for the determination of total serum copper[5] and antihyaluronidase[6] have been described. The latter is a modification of the mucin clot-prevention technique, with use of a commercial hyaluronidase obtained from bovine testicular tissue. Fresh unheated serum was employed in dilutions of 1:10, 1:50, 1:250, and 1:1250. Values are expressed in units equivalent to 0.04 turbidity-reducing units and as the reciprocals of the dilutions. This procedure has been shown to be capable of consistently indicating the difference between fivefold increments of inhibitor. Purified ceruloplasmin was prepared by the method of Holmberg and Laurell.[7]

Results

The results obtained from the study of human sera are presented in the Table. Total copper values were least in newborn babies, greater in normal adults, and highest in women in the last trimester of pregnancy. The hyaluronidase inhibitor content of the sera of these nondiseased persons was low, being 10 units in 12 and 50 units in 2, in spite of the fact that there was substantially more than a fivefold increase in total copper between the babies and the pregnant women.

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Dr. G. E. Cartwright, Dr. C. J. Gubler, and Dr. H. Markowitz, of the University of Utah, Salt Lake City, did the copper analyses and provided the supply of ceruloplasmin. The ceruloplasmin was isolated by Dr. Markowitz.

Antihyaluronidase and Serum Copper Levels in Various Conditions

Serum No.	Clinical Condition	Antihyaluronidase Titer, Units/Ml.	Serum Copper, μg./100 Ml.
1	Newborn	10	33
2	Newborn	10	42
3	Newborn	50	51
4	Normal adult	10	78
5	Normal adult	10	99
6	Normal adult	10	90
7	Carcinoma, lung	250	160
8	Leukemia	250	172
9	Pregnant	10	228
10	Pregnant	10	256
11	Pregnant	50	171
12	Pregnant	10	207
13	Pregnant	10	243
14	Pregnant	10	249
15	Pregnant	10	174
16	Pregnant	10	210

Two persons with advanced neoplastic disease exhibited the expected increase in hyaluronidase inhibition, and their serum copper values were also high.

Purified ceruloplasmin, 37.5 mg., was added to 1.0 ml. of normal Serum 6. The titer of hyaluronidase inhibitor was not increased above 10 units per milliliter in spite of an approximately one-hundred-fold increment in ceruloplasmin.

Summary

Total copper concentrations of sera obtained from healthy or pregnant human beings varied from 33μg. to 249μg. per 100 ml. of plasma in the absence of any related increase in nonspecific hyaluronidase inhibition as measured by the mucin clot-prevention test. Total copper reflects accurately the ceruloplasmin concentration, and these data indicate that this substance is not related to serum nonspecific hyaluronidase inhibitor.

This fact was confirmed when the addition of 100 times the normal concentration of purified ceruloplasmin to serum from a healthy human being failed to increase the titer of hyaluronidase inhibitor.

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Aortic Stenosis

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Progress in the development of surgical techniques for the treatment of aortic stenosis has provided a new challenge to physicians.[1-4] The increasing number of patients undergoing aortic valve surgery is a clear indication that management of patients with this lesion includes the evaluation and selection of candidates for surgical intervention.[5-8]

Experience in other forms of operable heart disease has shown that the proper selection of patients for surgery depends on the fulfillment of three requirements: (1) that the descriptive aspects and life history of the disease be well established; (2) that the pathologic physiology of the lesion be known; (3) that a quantitative estimate of severity can be made, either clinically or with the aid of special techniques.

The first requirement is abundantly fulfilled by the many excellent studies dealing with the clinical aspects and clinicopathologic correlations in aortic stenosis.[9-12] Most of these studies, however, were not oriented to the selection of candidates for surgery. Furthermore, clinical evaluation is limited, since some of the clinical manifestations in patients with aortic stenosis may be duplicated by other associated conditions, especially coronary artery disease,[9] and because of the frequent existence of associated valve lesions. The pathologic physiology of acute experimental aortic constriction has been studied in animals,[13] but until recently, knowledge of the disease in man was gained by translation and inference, with all their potential hazards. The naturally occurring disease is different in many important respects from the experimental lesion, partly because left ventricular hypertrophy and other as yet unknown compensations gradually develop. Knowledge concerning the pathophysiology of aortic stenosis in human beings is scanty. Without it, there have been no means of determining the severity of aortic obstruction.

Until recently, there has been no suitable way of studying aortic stenosis short of cardiotomy or autopsy. Right heart catheterization has been of great value in obtaining direct evidence of lesions involving the pulmonary vasculature and right heart and even in providing evidence of lesions of the left heart. Although studies utilizing this technique have been reported,[14] it is generally recognized that aortic valve disease cannot be evaluated critically by venous cardiac catheterization.[15] Gorlin et al.[15] studied patients with aortic valve disease by means of venous catheterization and later perfused the same hearts post mortem by the McMillan pump and made the most definitive analysis as yet available for the understanding of the disturbed physiology in the human disease. The introduction and development of left heart catheterization [16-21] as a practical method of determining pressures in the left heart has provided the essential tool for the extension of studies of aortic stenosis and for determining the severity of the lesion. Increasing numbers of

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studies utilizing this technique have appeared in the recent literature.[22-25]

The present report deals with clinical manifestations and findings by left heart catheterization in a group of patients with clinically "pure" aortic stenosis. Its purpose is to correlate the pathologic physiology of aortic stenosis with the clinical manifestations. To achieve this end with reasonable precision, it was deemed essential to exclude from the study all patients with any other valvular lesion.

Material and Methods

The clinical material consisted of 13 patients who were referred primarily for evaluation of aortic stenosis, with a view to possible surgery. Aortic stenosis was the sole valvular lesion in the entire group; one patient had mild aortic stenosis and probable severe coronary artery disease (Patient 13).

All patients had a systematic and detailed history and physical examination. Standard 12-lead electrocardiograms supplemented by two right chest leads (V_{4R} and V_{3R}) were obtained routinely. Vectorcardiograms and phonocardiograms were obtained in some instances. Fluoroscopy was performed, and roentgenograms were taken in the posteroanterior, lateral, and both oblique views; the cardiac size was determined by the standards of Ungerleider and Clark.[26] Chamber size was determined by a method described previously.[27]

Prior to left heart catheterization, the patient was given 0.1 gm. of pentobarbital by mouth and 0.064 gm. codeine by hypodermic. Before the patient was turned to the prone position, a 16- or 17-gauge Cournand needle was placed in the left brachial artery. Left atrial needle punctures were performed by one of us (D. E. H.) by the transthoracic approach of Björk as modified by Fisher, using a special 16-gauge thin-walled needle 19 cm. in length. A polyethylene catheter (Clay-Adams PE 60, 1.22 mm. O. D., 0.76 mm. I. D.) 43.5 cm. in length with an adaptor (Clay-Adams Size B plastic tubing to female Luer slip) on the proximal end was passed through the needle. Saran plastic tubing 3 to 5 ft. long was used to connect the catheter and brachial artery needle to Statham P23D strain gauge pressure transducers. Pressures were recorded on a Sanborn multichannel direct-writing oscillograph. Brachial arterial mean pressures were obtained by electrical integration; the left ventricular and brachial artery mean pressures during systolic ejection were obtained by planimetric integration of the systolic portions of the phasic records. The zero point for intrathoracic pressures was 10 cm. anterior to the skin of the back.

The polyethylene catheter was introduced through the needle in the left atrium into the left ventricle, with continuous monitoring of the pressure and electrocardiogram. Extrasystoles occasionally occurred and could be obviated by manipulation of the catheter. When the electrocardiogram was stable with the catheter in the left ventricle, simultaneous pressures were recorded from the left ventricle and brachial artery. The sensitivity of the manometer connected to the catheter was then suitably increased so that only the diastolic portion of the left ventricular pressure appeared on scale. A continuous record was then obtained while the catheter was withdrawn from the left ventricle to the left atrium and the presence or absence of a diastolic pressure difference across the mitral valve determined.

Immediately after these pressures were obtained, cardiac output was determined by the single-injection indicator-dilution method.[28] I^{131}-labeled human serum albumin was used as the indicator, and multiple samples from the brachial artery were collected in a rotating multiple sampling device at one-second intervals through a short segment of polyethylene catheter (PE 90). Each sampling tube contained a few particles of dried heparin. The timing of the injection and of the sampling tubes was recorded on the Sanborn Polyviso. The dead space of this collecting system was 1.0 ml., and flows ranged from 0.7 to 1.5 ml. per second. Three milliliters of I^{131}-labeled human serum albumin solution containing 5μc. to 10μc. per milliliter in a 5 ml. Gibson syringe was injected through the catheter into the left atrium in one second or less. The syringe and catheter were later calibrated by weight to determine the volume of solution delivered. Whole blood, 0.5 or 1.0 ml., was pipetted from each of the multiple samples, and the radioactivity was determined in a well-type scintillation counter for a sufficient duration to insure not more than 1% standard error. The injectate solution was diluted 1:1000, and duplicate 1.0 ml. aliquots were counted in a similar manner. After correcting for background counts and expressing the concentration in each sample in counts per minute per milliliter of whole blood, an indicator dilution curve was plotted on semilogarithmic graph paper and the cardiac output was calculated according to the following modification of the Stewart-Hamilton formula:

$$CO = \frac{60 \times V_1 \times C_1 \times 1000}{S}$$

where CO = Cardiac output in milliliters per minute
60 = Seconds per minute
V_1 = Volume of solution injected in milliliters
C_1 = Concentration of radioactivity in the diluted injectate in counts per minute per milliliter
1000 = Dilution factor of injectate
S = Sum of concentrations for each second from the beginning of the curve to that time on the extrapolated straight-line downslope at which the concentration is 1/100 the concentration at the beginning of the straight-line downslope in counts per minute per milliliter

Aortic valve stenotic areas were calculated by the Gorlin formula,[29] with use of the left ventricular pressure curve for measuring the actual systolic ejection period and 68.5 as the empirical constant. The calculation of total systemic resistance (TSR) and left ventricular work (LVW) have previously been described.[30] For the former, the brachial arterial mean pressure was employed, and for the latter calculation, the left ventricular mean systolic ejection pressure minus the left ventricular end-diastolic pressure was used as the pressure generated by the left ventricle.

In patients who subsequently underwent aortic valve surgery (performed by D. E. H.), the valve orifice was carefully palpated and a diagram was drawn from which the valve area was determined by planimetry. In patients coming to autopsy, the intact aortic valve was carefully examined and photographed. The size of the orifice of the aortic valve was determined by planimetry.

Results

The clinical data are listed in Table 1. By the New York Heart Association classification, one patient was in Class I; seven, in Class II; four, in Class III, and one, in Class IV. There were nine men and four women, ranging in age from 25 to 66 years, with an average age of 50 years.

Angina pectoris occurred in 9 of the patients; syncopal attacks, in 7, and exertional dyspnea, in 12. Orthopnea and paroxysmal nocturnal dyspnea were present in six patients.

The physical findings listed in Table 1 conform in every respect to those considered characteristic of the disease. The harsh, "diamond-shaped" systolic murmur of variable intensity with transmission into the neck, with or without a palpable thrill and diminished or absent aortic second sound, was uniformly present. In one case, the aortic second sound was of normal intensity, and in one it was accentuated. In no case was there auscultatory evidence of mitral valvular disease or of aortic insufficiency. The carotid pulses were plateau in character in 11 cases; the pulse was normal to palpation in 1, and in 1 no observation of the pulse was made. By the cuff method, the arterial pulse pressure was 30 mm. of Hg or less in only five patients. Despite frequent history of respiratory distress, rales

TABLE 1.—*Clinical Findings in Pure Aortic Stenosis*

Patient	Sex	Age	N. Y. Heart Assoc. Classification	Symptoms *					Aortic Second Sound †	Aortic Stenosis Murmur ‡	Carotid Pulse §	Cuff Blood Pressure		Rales in Lungs*	Electrocardiogram ‖
				Angina	Syncope	Dyspnea	Orthopnea	Parox. Noct. Dyspnea				Systolic	Diastolic		
1	F	60	III	+	+	+	0	0	−2	III	P	124	80	0	LVH
2	M	54	III	+	+	+	+	+	0	III	P	110	80	0	LVH
3	F	39	II	+	+	+	0	0	0	V	P	96	84	0	LVH
4	F	52	II	0	0	+	0	+	−1	III	N	146	98	+	LVH
5	M	25	I	0	0	+	0	0	+2	IV	P	125	105	0	LVH
6	M	53	II	0	0	+	+	+	−2	IV	..	130	70	0	LVH
7	F	57	IV	0	0	+	+	+	0	III	P	150	90	+	LVH
8	M	51	II	+	+	+	0	0	0	IV	P	110	80	0	LVH
9	M	66	III	+	0	+	+	+	0	II	P	125	85	0	LVH
10	M	40	II	+	0	+	0	0	N	III	P	120	80	0	LVH
11	M	44	II	+	+	+	+	0	−1	III	P	96	64	0	N
12	M	63	II	+	+	0	0	0	0	III	N	132	92	0	LVH
13	M	46	III	+	+	+	+	+	−1	III	P	120	78	0	LBBB

* + indicates present; 0, absent; —, no observation.
† Graded on basis of + (accentuated), N (normal), — (diminished), 0 (absent).
‡ Graded on basis of I to VI.
§ N indicates normal; P, plateau.
‖ LVH indicates left ventricular hypertrophy; LBBB, left bundle-branch-block. All patients had normal sinus rhythm.

TABLE 2.—*Radiological Findings in Pure Aortic Stenosis* *

Patient	Heart Size	Left Ventricle	Left Atrium	Pulmonary Vascular Markings	Aorta	Calcification of Aortic Valve
1	+5	1+	N	N	1+	+
2	+20	2+	N	2+	N	+
3	N	1+	N	1+	N	+
4	+20	1+	1+	1+	N	+
5	N	1+	N	1+	1+	+
6	+20	2+	N	2+	N	+
7	+26	2+	N	1+	1+	+
8	+20	1+	N	N	N	+
9	+25	3+	N	1+	1+	+
10	+20	2+	N	N	N	+
11	+5	1+	N	N	N	0
12	N	1+	N	N	N	+
13	+18	1+	N	N	N	0

* Heart size by height-weight standards of Ungerlieder and Clark, [26] N to 4+, chamber size described by Healey et al. [27] Calcified valve: 0 indicates absent; +, present. The main branch of the pulmonary artery and right ventricle appeared of normal size in all cases.

were found at the lung bases in only three patients. Peripheral edema, hepatomegaly, and distended neck veins were noted in none.

The electrocardiogram showed changes characteristic of left ventricular hypertrophy by the criteria of Sokolow [31] in 11 of the 13 cases. One patient (Patient 11) had no ventricular hypertrophy and one (Patient 13) had complete left bundle-branch block. All patients had normal sinus rhythm.

The radiologic findings are summarized in Table 2. Chamber enlargement was generally limited to the left ventricle and was surprisingly slight, even in the face of severe left ventricular hypertension and clinical and hemodynamic evidence of left ventricular failure. Heart size varied between 0 and +26% of predicted, with an average of +14%. The main trunk of the pulmonary artery and the right ventricle were normal in size in all cases. Unlike the poststenotic dilatation of the pulmonary artery in patients with pulmonic stenosis, the ascending aorta did not appear to be dilated in the majority of these patients, even though the aortic stenosis was severe in all but a few cases. In four patients, however, the ascending aorta did appear to be somewhat dilated and tortuous. The pulmonary vascular markings varied from normal in six to moderately prominent in seven patients. Eleven patients had visible calcification of the aortic valve at fluoroscopy. Calcification was not observed despite careful search in the other two patients. These two patients had the lowest left ventricular systolic pressures (178 and 111 mm. of Hg), while those with calcification had pressures above 185 mm. of Hg (Table 3, pressure values).

Physiologic Study.—The hemodynamic data are presented in Table 3. With one exception (Patient 9), the cardiac index was normal, averaging 4.2 liters per minute per square meter, with a range of 2.6 to 7.6 liters. The normal resting nonbasal cardiac index in this laboratory under conditions of right heart catheterization is 4.2 liters per minute per square meter, with a range of 2.8 to 5.0 liters. The finding of normal or elevated cardiac output was also found at right heart catheterization preceding the left heart or by cardiac outputs determined on different days from peripheral vein indicator injections. The stroke index in eight of these patients was within the normal range (over 35 cc. per beat per square meter). In five patients, however, it was below this value, but four of the five had tachycardia with a rate of 100 or more at the time. The fifth patient was Patient 9, whose minute cardiac index was slightly below normal (2.6 liters per minute per square meter).

Left ventricular systolic pressure averaged 217 mm. of Hg (range, 111 to 320) and was more than 185 mm. of Hg in 11 of the 13 cases. The high systolic pressures were maintained even in the face of elevations of left ventricular end-diastolic pressures above an arbitrary upper normal limit of 10 mm. of Hg in five patients. Systemic arterial pressure was generally normal. The systolic pressure was below 110 mm. of Hg in five but was over 145 mm. of Hg in three. The highest pressure in this group was 152/88 mm. of Hg. The mean systolic pressure difference across the aortic valve averaged 77 mm. of Hg (range, 24 to 150) and was 50 mm. of Hg or more in 11 cases. Mean pressure differences of 50 mm. of Hg or less were encountered in only two of

TABLE 3.—*Hemodynamics in Pure Aortic Stenosis*

Patient	Body Surface Area, Sq. M.	A-P Diameter of Chest, Cm.	Cardiac Index, L./Min./Sq. M.	Pulse Rate/Min.	Stroke Index, Cc./Beat/Sq. M	Pressures, Mm. Hg.							Mean Difference Across Aortic Valve During Systole, Mm. Hg.	Brachial Artery Systolic Upstroke Period, Sec./Beat		Total Systemic Resistance, Dynes/Sec./Cm.$^{-5}$ *	Left Ventricular Work, Kg. M./Min./Sq. M.	Left Ventricular Systolic Ejection Period, Sec./Beat	Size of Aortic Valve Orifice, Sq. Cm.		
						Left Ventricle			Brachial Artery												
						Systolic	End-Diastolic	Systolic Mean	Systolic	Diastolic	Systolic Mean	Mean		Observed	Predicted				Calculated *	Surgical Drawing	Autopsy
1	1.61	19.5	3.3	130	25	265	7	210	127	82	113	96	97	0.13	0.09	1,480	9.6	0.24	0.3		0.4
2	1.78	23.5	3.6	105	34	254	20	210	117	80	103	91	107	0.16	0.10	1,140	9.8	0.25	0.3		
3	1.62	19	3.9	120	33	220	7	175	94	64	83	75	92	0.14	0.09	940	9.4	0.25	0.3	0.4	
4	1.55	19	7.6	136	56	320	20	278	152	88	126	112	150	0.13	0.09	760	28.1	0.27	0.4		
5	2.06	20	2.9	81	36	200	14	160	100	74	90	84	70	0.16	0.11	1,120	6.1	0.31	0.4	0.9	
6	1.74	22	4.9	88	56	264	32	222	120	66	103	84	119	0.22	0.11	820	13.4	0.30	0.4	0.5	
7	1.75	20	4.1	102	40	242	10	190	130	86	120	108	70	0.16	0.10	1,200	10.6	0.26	0.5		
8	1.82	21	3.2	100	32	189	6	164	121	85	108	103	56	0.20	0.10	1,420	6.8	0.22	0.5	0.5	
9	1.94	20	2.6	95	27	188	18	158	123	71	105	90	53	0.16	0.10	1,440	5.2	0.26	0.6	0.6	
10	1.95		3.4	72	47	186	4	154	101	74	90	82	64	0.17	0.12	990	7.3	0.26	0.6		
11	1.79	18	4.8	106	45	178	3	149	106	62	90	78	59	0.16	0.10	730	10.1	0.24	0.6		
12	1.88	22	3.9	104	37	198	6	170	146	92	125	117	35	0.16	0.10	1,160	9.2	0.25	0.7	0.7	
13	1.84	21	5.0	82	55	111	6	97	84	62	73	70	24	0.20	0.10	600	6.5	0.25	1.4		

* Calculated by a modification of the method of Gorlin and Gorlin.[11]

these patients—one with aortic stenosis of only moderate severity (Patient 12) and one with mild aortic stenosis and presumably severe coronary disease (Patient 13).

The systolic upstroke time of the arterial pressure pulse was variably prolonged in each case, averaging 166% of predicted (range, 110% to 200%).

Calculated cross sectional area of the aortic valve in 12 of the 13 cases averaged 0.5 sq. cm. (range, 0.3 to 0.7). Patient 13 had a calculated valve size of 1.4 sq. cm. and was not included in this average because his disease was considered to be coronary artery sclerosis. The calculated and surgical or autopsy estimates of the cross sectional area of the orifice of the aortic valve are compared in Table 3 and illustrate good agreement in all but one instance (Patient 5).

There was no measureable pressure difference between left atrium and left ventricle during diastole in any of the patients except Patient 4. In this patient, there was a difference of 10 mm. of Hg associated with a cardiac index of 7.6 liters per minute per square meter. The calculated cross sectional area of the mitral valve was 5.0 sq. cm. Such a calculation is not justified under the conditions but indicates that mitral valve narrowing, if present at all, was so slight as to be of no significance. There was not even an apical diastolic murmur. Therefore, it was considered justified to include this patient in the group.

The average total systemic resistance, i. e., the resistance distal to the aortic valve, was 1064 dynes seconds centimeters^{-5} (range, 600 to 1480). All values were within the normal range.

Left ventricular work varied from the upper limits of normal to approximately six times the normal (Patient 4) as a consequence of left ventricular hypertension and normal flows. The average was about twice normal (10.2 kg. meters per minute per square meter).

Comment

Twelve of the thirteen patients in this study had pure aortic stenosis of severe degree. One additional patient with mild aortic stenosis and presumed advanced coronary disease was included. None of the patients had aortic insufficiency and none had mitral disease, except possibly Patient 4, in whom mitral stenosis was so mild as to be negligible. Because the material is limited and selective, this study provided an opportunity to correlate physiological with clinical findings at an advanced stage of the disease. It is unfortunate that earlier stages of aortic stenosis were not studied, but it was considered unjustifiable to perform left heart catheterization without therapeutic indications.

The cross sectional area of the orifice of the aortic valve in the average normal adult is 2.6 to 3.5 sq. cm.[32] As the aortic valve progressively narrows over the course of years, the circulatory changes are at first insignificant,[14] but eventually the valve becomes narrowed to the point where the hemodynamics are grossly altered. There are only two fundamental ways in which the heart can compensate for the obstruction. One is reduction in the amount of blood passing through the stenotic valve, i. e., reduced cardiac output; the second is an increase of systolic pressure in the left ventricle or a decrease in brachial arterial pressure. The relationship between the degree of stenosis, the pressure difference across the aortic valve, i. e., the left ventricular systolic mean minus the brachial arterial mean systolic pressures, and the amount of blood flowing through the valve during systole when the aortic valve is open has been described by Gorlin and Gorlin [29] as follows:

$$A = \frac{F}{K\sqrt{\Delta P}}$$

where A is the cross sectional area of the aortic valve, in square centimeters; F is the amount of blood flowing through the valve, in cubic centimeters per systolic second; K is an empirical constant (68.5), and ΔP is the mean pressure difference across the aortic valve during systole, in millimeters of Hg.

Harrison [33] was the first to report a normal cardiac output in an isolated case of aortic stenosis. Our study and others [14,15,34] indicate that in severe aortic stenosis cardiac output usually remains normal or may even be high. This remarkable finding was observed even in patients with an elevated left ventricular end-diastolic pressure, which is usually considered to indicate left ventricular failure. In many of our patients, direct Fick as well as dye outputs were performed in rapid succession and were in essential agreement. This finding of a normal cardiac output is quite unlike that occurring in other central obstructions of severe degree. For example, the cardiac output determined by the same methods and under the same circumstances of left heart catheterization used here in a group of patients with mitral stenosis has been characteristically low. Why the cardiac output is maintained at normal or even high levels in aortic stenosis at the expense of left ventricular hypertension is not immediately apparent, but this was the finding in 12 of the 13 patients.

If cardiac output remains normal, the pressure difference across the aortic valve must increase as the cross sectional area of the aortic valve becomes smaller. There is no measureable difference of pressure across a normal aortic valve. In fact significant obstruction to left ventricular emptying does not occur until the valve has narrowed to about 25% of its normal cross sectional area.[35] Progressive narrowing of the aortic valve toward 0.5 sq. cm.[14] or 0.6 sq. cm. (this study) is well tolerated because only modest elevations of left ventricular systolic pressures are required to maintain flow. As that critical narrowing of valve orifice is approached, however, slight decrements in valve size are associated with great increases of left ventricular systolic pressure.[14] In our cases with severe aortic stenosis the pressure difference was

increased to variable degrees, depending on the size of the orifice and the flow at the time. This increase of pressure difference is due almost entirely to an increase of left ventricular systolic pressure rather than to a fall of aortic (brachial arterial) pressure. Thus, the main consequence of aortic stenosis is left ventricular hypertension, not a reduction of cardiac output and not, in most cases, a fall of aortic pressure. The myocardium becomes markedly hypertrophied as a direct result of the ventricular hypertension. There is little evidence that the myocarditis associated with acute rheumatic fever in the remote past results in permanent damage to the myocardium. However, the compensatory powers of the left ventricle, no matter how remarkable, have limiting factors which may become apparent with the passage of time. Angina-like pain, syncopal attacks which may result in sudden death, and left ventricular failure may occur. These three complications of aortic stenosis form an ominous triad with a poor prognosis [9,11,12] and require special attention.

Angina Pectoris.—Angina pectoris or pain suggestive of angina [8,9] was present in 9 of our 13 patients. The precise mechanism underlying angina in aortic stenosis, uncomplicated by coronary disease, has never been established. The suggestions of relative myocardial ischemia resulting from (*a*) increased demands of the hypertrophied left ventricle and (*b*) the disproportion between left ventricular intramural and coronary artery pressure during systole are theoretically sound.[14,36]

It may be extremely difficult if not impossible to decide clinically whether angina pectoris is due to aortic stenosis or to associated coronary disease or to a combination of both factors.[10-12] In one patient (Patient 12) with angina and syncope but no heart failure clinically or physiologically, the calculated size of the aortic valve orifice was 0.7 sq. cm. Moderate coronary atherosclerosis was present at necropsy. Thus, both coronary artery sclerosis and severe aortic stenosis coexisted.

Another patient, Patient 13, had a Grade 3 murmur of aortic stenosis, an aortic second sound of diminished intensity, and the triad of angina, syncope, and left ventricular failure. There was left bundle-branch block found by electrocardiogram, and on fluoroscopy a cardiac silhouette was found which was exactly like that of the 12 other patients with severe aortic stenosis, except perhaps that no valve calcification was seen. The calculated size of the orifice of the aortic valve was 1.4 sq. cm.—an orifice size which of itself would lead to no disability. This patient's main lesion was, by exclusion, coronary disease and certainly not aortic stenosis. In this case coronary artery disease associated with mild aortic stenosis produced a clinical picture closely mimicking that of severe aortic stenosis.

Syncope.—Syncopal attacks occurred in seven cases. Their occurrence was not related to the level of the left ventricular systolic pressure, to the brachial arterial systolic or diastolic pressures, or to the magnitude of the cardiac output at the time of study. All of these patients also happened to have angina, but this of course is not a necessary relationship. The mechanism of syncope in aortic stenosis is unknown but is presumed to be on the basis of cerebral ischemia. It may be attributed to paroxysmal arrhythmias with a reduction of ventricular pressure and therefore of cardiac output, which in association with a severely stenotic aortic valve can be expected to produce a significant fall of arterial blood pressure. Another possibility is a fall in resistance in vascular beds other than the brain occasioned by exercise or emotion; this in association with a relatively fixed cardiac output would lead to diversion of blood away from the brain to the vasodilated parts.[10,12,14]

Left Ventricular Failure.—All but one of the patients complained of exertional dyspnea; six had orthopnea and attacks of paroxysmal nocturnal dyspnea, and three

had rales at the lung bases. Five had left ventricular end-diastolic pressures over 10 mm. of Hg at the time of study. Had the patients been exercised, the left ventricular diastolic pressures would undoubtedly have risen abnormally in many if not all of the patients.

The high systolic pressures that can be sustained at normal end-diastolic filling pressures are truly phenomenal and represent the physiologic advantages of ventricular hypertrophy. In this study, there seemed to be wide variations in the ability of the left ventricle to meet increased work requirements through hypertrophy without, in addition, an abnormal increase of end-diastolic pressure. Normal as well as increased end-diastolic pressures were recorded at left ventricular systolic pressures above 260 mm. of Hg (Patients 1 and 6) and at work loads of over 9 kg. meters per minute per square meter (Patients 1 and 2). Therefore factors other than pressure and work are involved in the production of failure. Atherosclerosis of the coronary arteries, presbycardia or the eventual wearing-out process of the myocardium, and hypertrophy outgrowing the coronary blood supply are some which come to mind.

Increases of left ventricular end-diastolic pressures are accompanied by similar rises of pressure in the pulmonary circuit. When these pressures exceed the osmotic pressure of plasma in the pulmonary capillaries, pulmonary edema ensues. It is usually not until then that the term "congestive" heart failure is applied clinically, even though the congestive aspects of failure occur much later than other physiologic abnormalities.[37] In any event, once overt congestive heart failure appears in aortic stenosis, the course is malignant, characteristically becomes refractory to medical therapy, and bears with it an average prognosis of about two years.[11,12]

From the foregoing discussion, it is concluded that left ventricular failure is a late manifestation of aortic stenosis and that syncope is probably a late manifestation. In the age group concerned, the association of coronary disease is so frequent that angina may occur with lower degrees of stenosis.

Murmur.—Although some authors[10,11] have suggested that the intensity of the murmur of aortic stenosis bears a close relationship to the severity of the lesion, this study confirms the findings of Mitchell et al.[12] that no good relationship exists. The intensity of the murmur varied from Grade 2 in Patient 9 to Grade 5 in Patient 3, both with aortic stenosis of extreme severity. Grade 3 murmurs occurred in patients with calculated aortic valve orifice areas varying from 0.3 to 1.4 sq. cm. The intensity of the murmur failed to correlate with the stroke output or with the velocity of flow through the valve during systole $\left(\frac{\text{flow per systolic second [cubic centimeters per second]}}{\text{cross sectional area of orifice [square centimeters]}}\right)$. Cases of severe aortic insufficiency with a murmur of aortic stenosis have frequently been observed with no pressure difference across the aortic valve. This is a common finding in syphilitic aortic insufficiency. Although the size of the orifice and the flow through it are factors in determining the intensity of the murmur, it is apparent that they are only two of several variables.

Aortic Second Sound.—The aortic second sound was diminished in intensity or absent in 11 patients. In one it was normal, and in one it was accentuated. In both, the valve was calcified by fluoroscopic examination and valvular stenosis was found at surgery. Unfortunately phonocardiograms were not recorded. The possibility exists that these sounds were due to pulmonic valve closure.

Abnormalities of Pulse.—Abnormalities of the peripheral pulse (plateau, parvus et tardus) have long been considered characteristic signs of severe aortic stenosis, and this is generally borne out in this study. Eleven patients had a so-called plateau pulse by palpation of the carotid arteries. Patient 4, with an aortic valve of 0.4 sq. cm., had a carotid pulse that was normal in every respect. In another patient (Patient 12) with a valve of 0.7 sq. cm., there was inequality of the two carotid pulses; one was

normal, and the other was small. Unfortunately, too few cases of mild to moderate aortic stenosis have been studied to ascertain at what stage of valvular narrowing these signs appear.

Blood Pressure.—Blood pressures were usually within the normal range. In two cases they were below 100 mm. of Hg systolic, and the highest was 152 mm. of Hg systolic. Severe hypertension, however, can occur in patients with aortic stenosis.[11,12] A pulse pressure of 30 mm. of Hg or less was found in only four of these cases as measured by either the cuff method or direct arterial pressure-pulse tracing taken at the time of left heart catheterization. It exceeded 50 mm. of Hg in two cases by the cuff method and in five by direct measurement. When narrow, it is a useful sign, suggesting severe aortic stenosis; unfortunately, as others have emphasized,[10-12] it is often normal when aortic stenosis is severe.

Electrocardiogram.—Left ventricular hypertension is the most important hemodynamic consequence of aortic stenosis and, when of sufficient degree and duration, leads inevitably to hypertrophy of the left ventricle. Left ventricular hypertrophy was present by electrocardiogram in 11 of the 13 cases. Patient 13, with associated coronary artery disease, had left bundle-branch block. In only one patient did the electrocardiogram fail to show left ventricular hypertrophy. This patient (Patient 11) had a calculated aortic valve area of 0.6 sq. cm., a left ventricular peak systolic pressure of 178 mm. of Hg, a cardiac index of 4.8 liters per minute per square meter, and a work load of 10.1 kg. meters per minute per square meter. Unfortunately it is impossible to conclude from this study at what point in the time course of this disease electrocardiographic evidence of left ventricular hypertrophy appears.

Radiology.—As the electrocardiogram reflects hypertrophy of the left ventricle, roentgenograms reflect largely its dilatation. The severity of aortic stenosis was poorly correlated with the radiologic picture. Eight patients had only slight (1+) enlargement of the left ventricle fluoroscopically, despite left ventricular systolic pressures over 200 mm. of Hg in four. Five had either normal or only slight cardiac enlargement. One patient (Patient 4) with a left ventricular systolic pressure of 320 mm. of Hg had a heart only 20% enlarged. There was a better correlation of left ventricular size with left ventricular end-diastolic pressure than with systolic pressure, but even here the correlation was imperfect, presumably due to the decreased distensibility of the hypertrophied left ventricular myocardium. In six of the eight patients with only 1+ enlargement of the left ventricle fluoroscopically, the end-diastolic pressure was within normal limits. In three of the five patients with left ventricles 2 to 3+ enlarged, the end-diastolic pressure was more than 10 mm. of Hg. Heart size followed this general pattern. It would appear that cardiac enlargement in aortic stenosis implies elevation of the left ventricular end-diastolic pressure.

The left atrium was of normal size radiologically in 12 of the cases, despite an elevated left atrial mean pressure in 2. In the other case there was 1+ enlargement associated with a left atrial pressure of 25 mm. of Hg. If the left atrium is enlarged, it suggests coexisting mitral valvular disease.

Mitchell et al.[12] related calcification of the aortic valve mainly to age. Our youngest patient with a calcified valve, however, was only 25 years old.

Thus, even in advanced aortic stenosis the radiologic appearance of the heart was of little help in determining the severity of the stenosis. The left ventricles were usually normal or only slight enlarged, even in the presence of marked left ventricular hypertension. At postmortem examination the walls of the left ventricles of two of these patients (Patients 1 and 12) were 18 and 17 mm. in thickness, respectively.

Brachial Artery Pressure Pulse.—Prolongation of the systolic upstroke time of the brachial arterial pressure pulse (period from the end of diastole to the peak of

systole) with or without anacrotism was found in all patients. It has been considered to be a characteristic sign of aortic valve obstruction, and varying degrees of emphasis have been placed on it.[15] The results of the present study cast doubt on its importance, since little correlation between the degree of stenosis and the duration of systolic upstroke was found. Wright [23] has shown that prolongation of the central pulse in aortic stenosis was best correlated with the stroke output. There was a similar trend in the present study, even at the distant brachial artery.

Anacrotism, either in the form of a shoulder or distinct notch, was an inconstant finding. There was no relation between its presence or magnitude and the degree of valve narrowing in these patients with chronic disease, in contrast to the findings in acute experiments in dogs.[13,38]

Evaluation of Aortic Stenosis by Left Heart Catheterization.—Generally there is an inverse correlation between the degree of valve narrowing and the square root of the pressure difference across the aortic valve, but the range is too great to permit the use of pressure alone in assessing the degree of stenosis. Two patients in this series, for example, with identical calculated aortic orifice areas of 0.6 sq. cm. (Patients 4 and 5), had peak systolic pressure gradients of 168 and 100, respectively, and mean systolic pressure differences of 150 and 70 mm. of Hg, respectively. The left ventricular systolic pressure and the pressure difference across the valve may vary considerably, depending upon both the valve size and the flow, and the flow will vary with the circumstances under which it is measured. This emphasizes the importance of measuring both pressures and flow together and relating them to one another. The value of determining valve area is that it does relate pressure to flow and remains constant despite changes in these two factors. It is apparent that the severity of aortic stenosis cannot be accurately delineated from measurement of pressures alone and that the flow across the valve must also be measured at the same time. If significant aortic insufficiency exists, it is necessary to measure regurgitant flow in addition to that which continues out the aorta, the sum of the two being the total flow through the aortic valve during systole. Since none of these patients had aortic insufficiency, this does not apply to this study.

Therapeutic Implications.—The aim of any rational approach to the problem of aortic stenosis must be the reduction of left ventricular hypertension, for it is this compensatory phenomenon which usually leads to disability and ultimate death. Considering the various factors in the Gorlin formula, it is apparent that this can be achieved, theoretically at least, in several possible ways: (1) enlargement of the stenotic orifice, (2) reduction of cardiac output, (3) lengthening of the period for systolic ejection, and (4) reduction of the aortic pressure.

Enlargement of the stenotic orifice is, of course, a surgical procedure and is the most direct approach to the problem. It is limited by the fact that the valve is likely to be so calcified and scarred in the adult form of the disease that relief of the obstruction and maintenance of good valve action are technically difficult. The problem of surgery for relief of aortic stenosis is much different from that of mitral stenosis. However, a considerable hemodynamic improvement has recently been shown by left heart catheterization studies before and after aortic valvuloplasty, and there have been only 3 operative deaths in the last 60 patients undergoing surgery.

The observation that cardiac output is maintained at normal levels in pure aortic stenosis provides a nonoperative therapeutic inroad not previously considered. Since in our experience the homeostatic adjustments to pure aortic stenosis do not include a reduction of cardiac output until almost agonally, it would appear rational to induce such a reduction by medical thyroidectomy with radioactive iodine, as has been advocated for severe angina pectoris.[39] Any decrease of output would be accompanied by

a much greater fall of left ventricular pressure because, from the relationships that exist, the flow at any given valve size is related to the square root of the pressure difference. However, until the efficacy of induced hypothyroidism in reducing the cardiac output in patients with aortic stenosis is established, such an approach must obviously be conducted at an experimental level.

The last two possibilities offer little promise of therapeutic application.

Summary

Thirteen patients with pure aortic stenosis have been studied clinically and hemodynamically by left heart catheterization.

In this study, the critical cross sectional area of the aortic valve was about 25% of the normal, specifically about 0.6 sq. cm. or less (normal size, 2.6 to 3.5 sq. cm.).

The cardiac output in aortic stenosis was almost uniformly normal or higher than normal, even in the face of a high left ventricular end-diastolic pressure, i.e., left ventricular failure. The reason is not readily apparent. This is in striking contrast to other central obstructions such as mitral stenosis, in which the cardiac output is almost uniformly reduced.

The main consequence of aortic stenosis was left ventricular systolic hypertension. The highest systolic pressure recorded was 320 mm. of Hg.

In these symptomatic patients, the average left ventricular minute work load was about twice normal and in one case was about six times normal.

This enormous work performance was possible through the medium of left ventricular hypertrophy, but this study did not reveal at what point in the course of aortic stenosis left ventricular hypertrophy by electrocardiogram appeared. All but one patient with a cross sectional area of the aortic valve of 0.7 sq. cm. or less had left ventricular hypertrophy electrocardiographically. Eventually, left ventricular failure occurred, as indicated by a rise of left ventricular end-diastolic pressure.

Since severe aortic stenosis occurs characteristically in the older age group, when coronary disease is common, it was often impossilbe to decide clinically whether angina was due mainly to coronary disease or to aortic stenosis. Only if severe aortic stenosis was found by left heart catheterization or if electrocardiographic evidences of myocardial infarction were present could a decision be properly made. To further the difficulty, however, considerable coronary disease and aortic stenosis may coexist.

Angina with less severe aortic stenosis was considered to be on the basis of coexisting coronary artery disease.

In one patient with coronary artery disease but only mild aortic stenosis, the clinical picture resembled that of severe aortic stenosis in that angina, syncopal attacks, and left ventricular failure were present.

Aortic stenosis often produced little radiological evidence of left ventricular enlargement, even when the left ventricular systolic pressures were very high, because hypertrophy is poorly reflected radiologically. Even with left ventricular failure, as indicated by a high end-diastolic pressure, there was often little left ventricular enlargement by x-ray, presumably because of the relative lack of distensibility of the heavily hypertrophied muscle.

Aortic stenosis produced little if any enlargement of the left atrium, even when there was left ventricular failure with increase of the left atrial pressure.

There was a poor correlation between the severity of aortic stenosis and the pulse pressure, duration of the brachial arterial systolic upstroke, and anacrotism. Although a plateau pulse of the carotid artery was found almost uniformly at the advanced stage under study, it was absent in one patient with severe stenosis and present in one with mild stenosis.

The evaluation of the severity of aortic stenosis before the advent of syncope, angina, or left ventricular failure has therefore

not been found easy by clinical methods, except by the use of left heart catheterization. With use of this technique, both pressure and flow must be measured in order to ascertain the size of the orifice of the aortic valve, either one alone being potentially misleading in its assessment. With knowledge of the flow and the pressure difference across the aortic valve, its cross sectional area was found to be calculable by the Gorlin formula with a high degree of accuracy.

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Transmission of the Common Cold to Volunteers Under Controlled Conditions

I. The Common Cold as a Clinical Entity

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The common cold is one of the most frequent and universal infections of man. Nevertheless, little is known regarding its specific etiology, and the diagnosis must be a clinical one. Until the infectious agent can be propagated, characterized by microbiologic and pathologic methods and consistently transmitted to experimental animals or in tissue cultures, transmission to humans appears to be the best method for study. In this manner, information can be obtained about the nature of the infection, factors that influence the susceptibility or resistance of the human host, and conditions that favor spread of the illness.

During the past five years we have made observations upon more than 1000 volunteers who were challenged with infectious nasal secretions obtained from persons with a common cold or with a blank solution used as a control. The clinical findings were recorded daily. Examination of the nose and throat was of no value in detecting the presence of a cold, and it was necessary to develop an objective and consistent method of scoring symptoms. Upon this basis, criteria were developed for the diagnosis of a cold following challenge with an infectious secretion which permitted the uniform evaluation of serial experiments. The present report describes the clinical features of the common cold among volunteers and the criteria that were used to diagnose an experimental cold.

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† Dr. Spiesman died Sept. 11, 1954.

Methods

Nasal secretions were obtained from a donor with a typical common cold and filtered through a bacteriologic porcelain filter. The specimen was diluted 1:10 with an isotonic salt solution buffered to pH 7.4 to which 0.5% yeast extract or 5% human Type O hemoglobin was added as a stabilizing protein. Multiple aliquots were stored in screw cap vials at —70 C in a mechanical refrigerator. Samples of the nasal secretion were routinely inoculated into embryonated hen eggs by both the allantoic and the amniotic routes, for the identification of influenza, mumps, and psittacosis viruses. Suckling mice were given intraperitoneal injections, and adult mice, both intraperitoneal and intracranial, for the identification of Coxsackie, herpes, and encephalitis agents. Human epithelial cells, strain HeLa, and monkey-kidney cells in tissue cultures were used to identify adenovirus and poliomyelitis viruses. Suckling hamsters were given inoculations of some of the secretions. Serial passages, animal fatality, hemagglutination, cytotoxicity, histocytology, and serologic methods were used to identify, insofar as possible, strains of known viruses. Secretions that were found to be free of disease-producing agents were tested for infectivity in from 6 to 12 volunteers.

Aliquots of an infectious secretion were thawed immediately before use in individual experiments and again diluted 1:10 with a buffered isotonic salt solution, which made the final dilution of the nasal secretion 1:100. Inoculations were made by instilling 0.2 ml. of the solution into each nostril of the volunteer while he was in a supine position. The blank control for these secretions was an identical solution of yeast extract or Type O hemoglobin in buffered salt solution.

The experimental subjects were all young adults, most of whom were between the ages of 20 and 25. The entire range of ages was 18 to 48 years. The majority were students in nearby schools of medicine, dentistry, or nursing. All of them were in good health and had been free from respiratory illness for at least six weeks prior to challenge. During the week before the experiment each person completed a questionnaire regarding his susceptibility to respiratory infections, the usual manifestations of his spontaneous colds, allergies, and certain relevant personal habits. A miniature chest roentgenogram also was made. Immediately prior to the experiment each person had an examination of his nose and throat. The color of the nasal mucosa was estimated by comparison with a color dictionary[1] in which 11 gradations of colors were used. Turgesence of the inferior turbinates and the amount of secretion were estimated on a scale of 1 to 4+. A record was made of coryzal or respiratory symptoms that were characteristic of the individual person or present on the day of challenge. A specimen of blood was drawn from each subject, and he was assigned to an experimental group composed of 5 to 20 volunteers.

Following the challenge the subject returned on the second, third, fourth, and sixth days to report his symptoms to an observer who was not informed regarding the nature of the challenge. Eight symptoms characteristic of the common cold—sneezing, headache, malaise, chilliness, nasal discharge, nasal obstruction, sore throat, and cough—were graded from 0 to 3+, according to their absence or presence in mild, moderate, or severe degree and recorded for each 24-hour period after the experimental challenge for six days. At the conclusion of the experiment any increase in the eight symptoms was observed, and the total symptom score during the six-day period was determined by adding the number of plusses above the baseline of symptoms reported by the volunteer for the day prior to challenge. Daily examination of the nose and throat was done in the early experiments. Bacteriologic cultures and other special procedures were done according to the experimental protocol. Ten to twenty-one days after the challenge a second blood specimen was drawn for serologic tests.

Results

Development of Criteria for the Presence of an Experimental Cold.—Since completely objective measures of the presence or absence of a cold were not available, it became necessary to develop suitable criteria which were not subject to biased manipulation by the investigators. Analysis of the frequency distribution according to the total symptom score of infected and noninfected volunteers showed the ascendancy of the former population at a score of 13. From this point forward and backward the two populations were statistically significantly different. Therefore a symptom score of 14 or more was established as the first criterion for the presence of an experimental cold.

The use of a single rigid criterion, however, significantly prejudiced the data by the exclusion of persons who subjectively developed a cold with fewer than 14 symptom points, a situation that occurred in nearly one-fourth of the experimental colds, and by the inclusion of a few persons who developed symptoms from other causes. Hence, a second criterion for an experimental cold was the impression of the subject that a common cold had developed during the period of observation.

Since the subjective evaluation of a cold also was not considered entirely reliable,[2] a third criterion for the volunteers with a subjective cold but fewer than 14 symptom points was the presence of increased nasal discharge on three or more of the six days after the experimental challenge. This excluded the majority of "overcooperative" volunteers who tended to diagnose a cold on the basis of a few bizarre symptoms. Subjects with more than 14 symptom points also were required to meet one of the other two criteria in order to exclude the symptomatic noncold group.

The frequency distribution of the volunteers who did or did not develop an experimental cold by these criteria is shown in Figure 1. The characteristics of the two curves suggest distinct populations, with independent normal distributions with re-

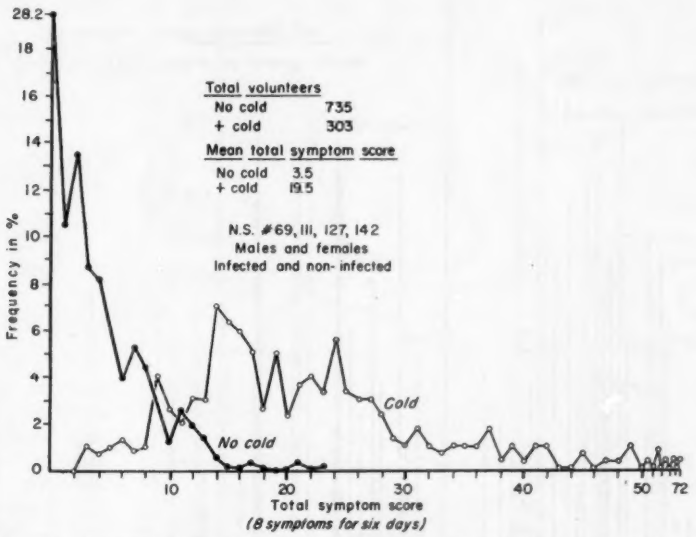

Fig. 1.—The frequency distribution by symptom score of volunteers who developed or did not develop an experimental common cold.

spect to the symptom score, which supports the validity of the criteria used and the advantage of the separate but interrelated criteria for the presence of a test cold.

Susceptibility of Volunteers to an Infectious Challenge.—The susceptibility of volunteers to four infectious secretions which were collected from different donors in four successive years is shown in Table 1. The per cent of volunteers who developed an experimental cold following challenge with the different secretions is surprisingly constant and suggests that the natural susceptibility of volunteers to most infectious secretions under the conditions of these experiments is approximately 35% to 40%. Females were significantly more susceptible than males to only one of the four secretions, and the over-all difference in the susceptibility between the sexes was slight. Spontaneous colds occurred in nearly 10% of the control group, but with each secretion the difference in the number of colds among the infected volunteers and their corresponding controls was statistically highly significant.

Experiments have been performed in every season and in 36 of the calendar weeks of the year. No particular influence of the season of the year was observed upon the susceptibility of volunteers to experimental colds, although the variation in different experiments was appreciable.

TABLE 1.—*Infectivity of Different Nasal Secretions*

Nasal Secretion No:	Males		Females		Total *	
	No. Challenged	% Colds	No. Challenged	% Colds	No. Challenged	% Colds
69	165	33	52	48	217	35.5
69 controls	62	8	27	4	89	6.7
111	55	38	127	35	182	36.2
111 controls	29	7	56	16	85	11.8
127	164	36	129	36	293	36.0
127 controls	48	10	37	8	85	9.4
142	116	39	64	52	180	43.0
142 controls	21	14	35	6	56	9.0
Total challenged	500	36.0	372	40.2	872	38.8
Total controls	160	9.4	155	9.7	315	9.5

* The differences in the susceptibility between males and females are not statistically significant except for NS No. 69, $P=0.05$.

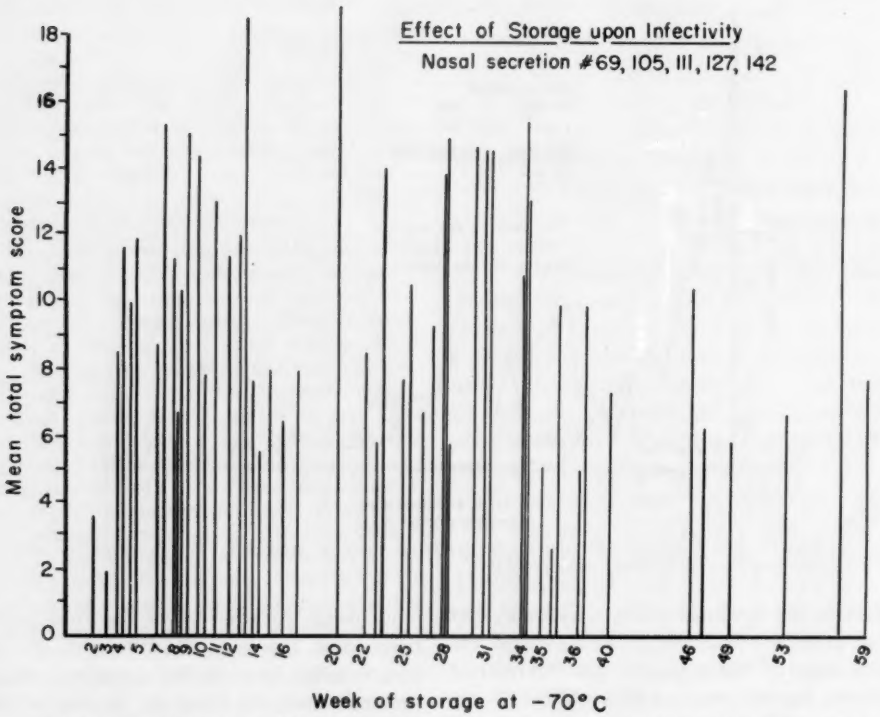

Fig. 2.—The infectivity of common cold nasal secretions after variable periods of storage at −70 C.

The effect of the storage of the nasal secretions at −70 C upon their ability to produce symptoms in volunteers was observed for periods up to 59 weeks. The accumulated data are illustrated in Figure 2 without regard for the nature of the individual experimental protocols, the season of the year, or the specific secretion. It is apparent from the graph that the infectivity is maintained fairly well. The analysis of individual secretions for infectivity after storage, however, suggests that one, Secretion 105, which has been excluded from experimental use, definitely lost potency during storage for 5 to 10 months. With one possible exception, the other secretions showed no loss of infectivity during periods of storage for 40, 48, 53, and 59 weeks during which they were used.

The Clinical Syndrome of the Experimental Cold.—The relative occurrence among infected and noninfected volunteers of four symptoms that were early manifestations of infection with a common cold agent is shown for successive days after challenge in Figure 3. Headache, sneezing, chilliness, and sore throat became prominent quickly after infection and also declined rapidly after one or two days' duration. The other four symptoms that were monitored—malaise, nasal discharge, nasal obstruction and cough—developed less rapidly and were more persistent, as shown in Figure 4. The significance of the difference in the occurrence of individual symptoms among the infected and noninfected volunteers on different days after the experimental challenge with one infectious secretion is given in Table 2. The symptoms are listed according to their general order of appearance among infected volunteers. The values for the significance of the symptoms traces the character and progression of the experimental illness.

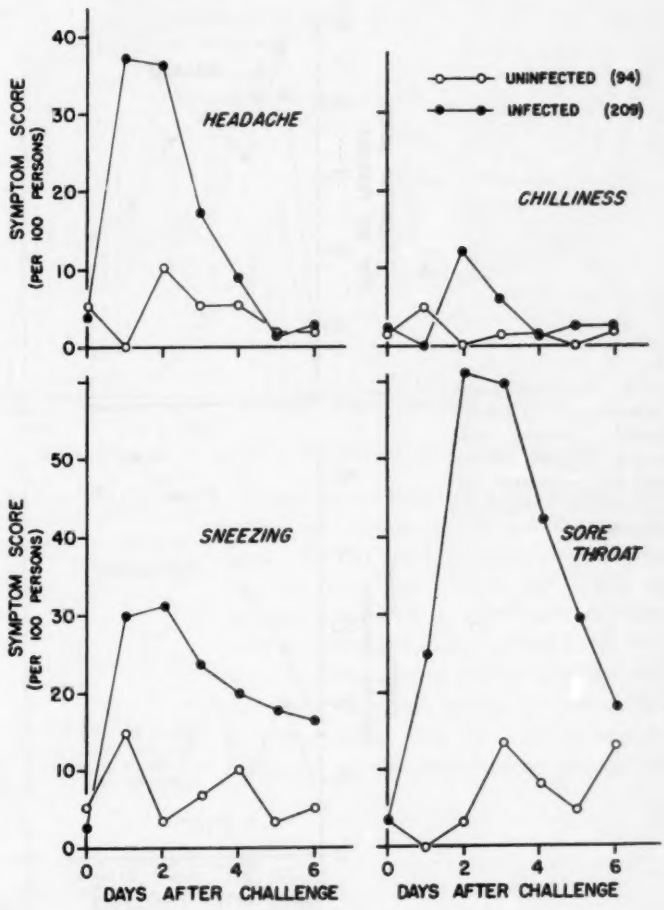

Fig. 3.—The occurrence of four early symptoms of an experimental common cold among infected (Secretion 69) volunteers and concomitant uninfected controls.

The similarity and relative severity of the individual symptoms produced by different infectious common cold secretions from four donors are shown in Figure 5. Sneezing and sore throat were the most prominent early symptoms. Nasal discharge and nasal obstruction were the severest of all the symptoms and between them accounted for nearly one-half of the total symptom score among volunteers regardless of which secretion caused the illness. Among males, nasal discharge, nasal obstruction, and malaise comprised a relatively greater proportion of the experimental illness, whereas females reported more sneezing, headache, and cough. The mean total symptom score among males who developed a cold was 20.1 and among females, 23.4.

A comparison of the over-all severity of the illness produced among volunteers by each of the four infectious secretions is given in Table 3. The comparison is based upon the mean total symptom score for the entire group of volunteers who developed colds after challenge with each of the secretions. A mean symptom score of 19.9 to 24 was characteristic of infection with any of the agents. Translated into illness this is equivalent to one day of mild headache, chilliness, and malaise and two days of moderately severe sore throat and sneezing, severe nasal discharge, moderately severe

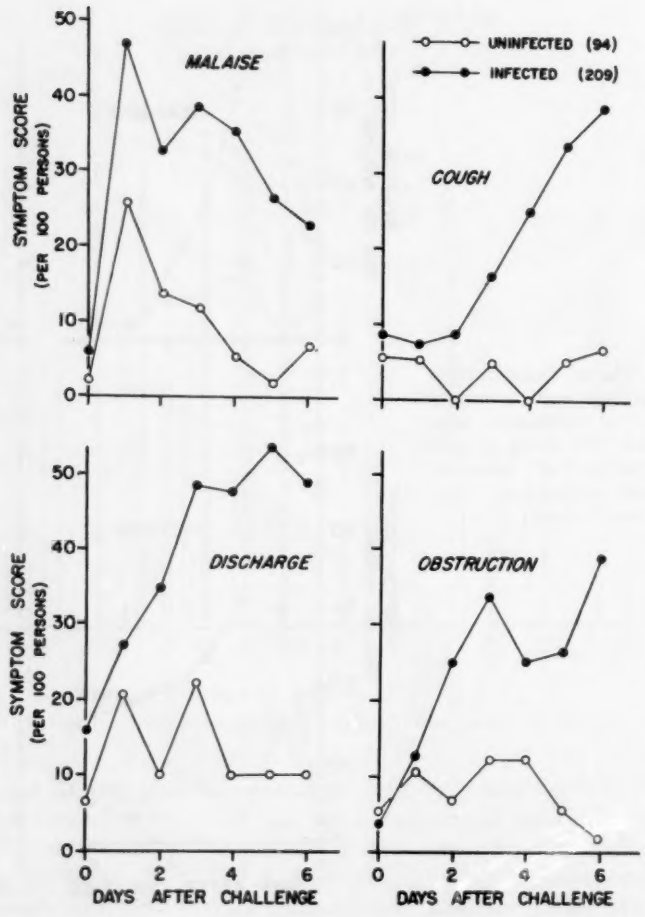

Fig. 4.—Four later symptoms of an experimental common cold among infected (Secretion 69) volunteers and concomitant uninfected controls.

TABLE 2.—*Significance of the Difference in the Occurrence of Symptoms Among Infected and Noninfected Volunteers*

Symptom	*P** Day After Challenge (Nasal Secretion 69)					
	1	2	3	4	5	6
Headache	0.01	0.01	0.05	NS	NS	NS
Sneezing	0.05	0.001	0.01	NS	0.05	NS
Chilliness	NS†	0.01	NS	NS	NS	NS
Sore throat	0.01	0.001	0.001	0.001	0.05	NS
Malaise	0.01	0.05	0.01	0.001	0.001	0.05
Nasal discharge	NS	0.01	0.05	0.001	0.001	0.001
Nasal obstruction	NS	0.05	0.05	NS	0.01	0.001
Cough	NS	0.05	NS	0.001	0.001	0.001

* *P* indicates statistical probability that the difference might be due to chance was less than the fraction noted.
† NS indicates the difference was not statistically significant at the 5% level.

nasal obstruction, and mild to moderate cough within the six-day period of observation after challenge. In general, the experimental illness caused more symptoms than the spontaneous colds observed among noninfected volunteers during the same time, but the latter often did not have their full course during the period of observation. Volunteers who were free from colds after the experimental nasal instillation had a mean symptom score of only 1.9 to 4.0, which was comparable to noninfected volunteers and equivalent to only one day of moderate headache or headache and malaise, possibly caused by the experiment per se rather than infection.

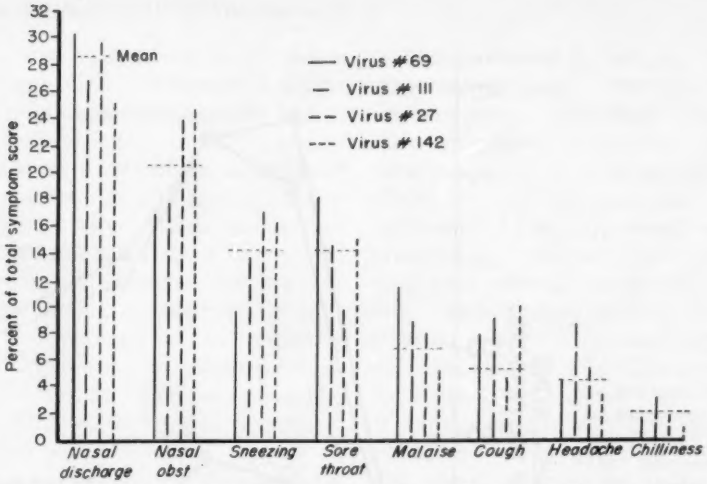

Fig. 5.—Symptoms of experimental colds produced by different infectious nasal secretions.

TABLE 3.—*Average Severity of Experimental Colds*

Nasal Secretion No.	Mean Total Symptom Score of Volunteers *			
	Infected		Noninfected	
	Developed Cold	No Cold	Developed Cold	No Cold
69	21.3	3.9	15.3	2.4
111	24.0	4.4	14.9	3.8
127	19.9	3.3	22.3	4.0
142	24.0	2.8	14.0	1.9

* Average increase in eight symptoms recorded daily for the 24-hour period before challenge and 6 days after nasal instillation. The number refers to the sum of the mathematical values used to denote the presence and severity of symptoms: 0 indicates none; 1, mild; 2, moderate; 3, severe.

Experimental infection with common cold agents was almost never accompanied by fever. In fact, oral temperatures taken at 7 a. m., noon, and 7 p. m. for groups of volunteers showed a significant displacement downward during the first three days after challenge among infected persons who were later classified among the group that developed colds (Fig. 6). There was no difference in the observations among in-

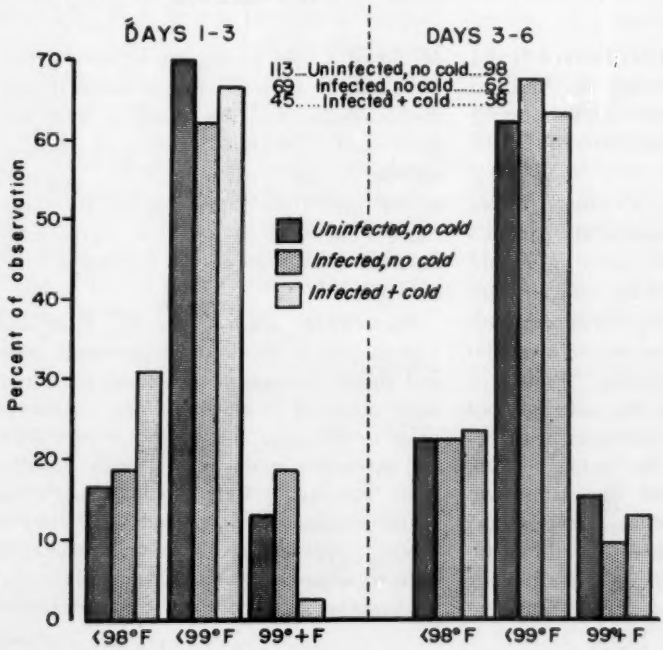

Fig. 6.—The distribution of measurements of oral temperature of experimental volunteers taken morning, noon, and evening each day after nasal instillation.

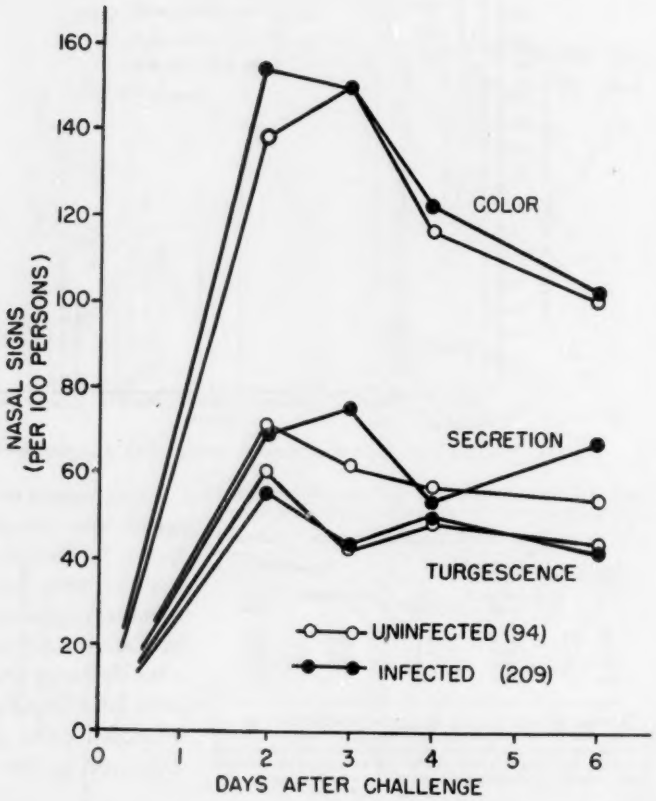

Fig. 7.—Comparison of nasal signs determined by examination among infected (Secretion 69) and noninfected volunteers.

fected volunteers who did not become ill and noninfected subjects. During the third to sixth days the results were similar among all three groups and temperatures of 100 F or more were rare.

Nasopharyngeal signs, determined by an examiner who was not aware of the patient's symptoms and uninformed as to the nature of the instillation given to the subject, were of no aid in distinguishing volunteers with a cold except in the occasional subject who had grossly obvious coryza. Figure 7 shows the similarity of the infected and noninfected groups of volunteers with regard to the color of the nasal mucosa, turgesence of the inferior turbinates, and the amount of nasal secretion as determined by examinations of the nose and throat on the second, third, fourth, and sixth days after the challenge. If only the subjects who developed a cold among the infected group were considered in the comparison or if only the examination on the second or third day were used a relative increase in redness, turgesence and secretion were observed among the infected persons but the mean changes were slight and not significantly different from noninfected volunteers without colds.

Bacteriologic Cultures of the Nose and Throat.—In a series of experiments nose and throat cultures for bacteriologic study were obtained from all of the volunteers prior to challenge and on the second, fifth, and seventh to ninth days after the instillation. The results were tabulated according to the incidence of β-hemolytic Staphylococcus pyogenes var. aureus, β-hemolytic Streptococcus pyogenes, Hemophilus, and coliform bacilli. The initial cultures showed

the incidence of staphylococci in the nose to be 40% and 47%, respectively, in the groups given infectious secretions and buffer; in the throat it was 25% and 13%, respectively. Only one-fourth of the strains were coagulase-positive. Beta-hemolytic streptococci (all groups) were present in the noses or throats of 13% and 11% of the test and control groups. Hemophilus (all species) were present in the nasopharynx of 12% and 15% of persons in the respective groups. The frequency of coliform organisms was negligible. Pleuropneumonia-like organisms were searched for in some nasal secretions but were not found.

The presence of these bacterial strains was strikingly similar among the infected and noninfected groups throughout the subsequent seven days without any increase in their prevalence, although more than one-half of the subjects given the infectious secretion developed symptoms of a cold. No type of organism was observed in more than 37% of persons who developed colds, and none of the genera studied appeared to prevent the development of a cold in some persons who harbored the species. Among persons who had staphylococci or streptococci in their throats at the time of instillation, the occurrence of cold symptoms after challenge with the infectious secretion was slightly greater than among the group as a whole. Similarly, the per cent of persons who harbored streptococci on the second and fifth days after infection was greater among persons with colds than among persons without colds, whether they received virus or buffer. None of these differences was statistically significant.

The Incubation Period and Duration of Experimental Colds.—Although the four infectious agents that were collected from different donors in successive years have been shown to be strikingly similar with respect to their infectivity and the nature and severity of the experimental illness they produced in volunteers, interesting variations were observed in the time after challenge, when a sharp increase in symptoms occurred among infected volunteers. These differences are noted in Table 4, which gives the mean daily symptom score for groups of subjects infected with each agent. All of the groups of subjects were comparable with regard to symptoms at the beginning of the experiment. Also, there was a mean increase in symptoms within 24 hours after the instillation of any of the secretions into the nares of susceptible subjects, which could have been caused in part by the experimental procedure.

Secretion 69, collected in 1953, caused the greatest mean increase in symptoms on the first day after challenge and on successive days a progressive increase in symptoms, which reached a peak on the third day. By the sixth day after the instillation the volunteers were nearly free of symptoms. In contrast Secretion 111, collected in 1954, caused no increase in symptoms among infected volunteers from the first to the third days after instillation and then produced a generally severer illness, which reached its climax on the sixth day. The action of the other two agents, NS No. 127 and NS No. 142, was intermediate between these two, but they also differed from each other in the time characteristics of the symptoms

TABLE 4.—*Average Incubation Periods of Experimental Colds*

Nasal Secretion No.	No. Colds	Mean Symptom Score of Volunteers * Day 0	1	2	3	4	5	6	Total
69	78	0.3 †	2.5	4.6	5.1	4.3	3.7	0.9	21.3
142	30	0.4	1.4 †	2.9	5.0	5.2	4.9	4.1	24.0
127	86	0.4	1.2	1.7 †	3.6	5.6	4.9	4.1	19.0
111	60	0.5	1.9	1.5	1.9 †	3.9	6.6	6.8	24.0

* Average daily increase in eight symptoms recorded for the 24 hours prior to challenge and 6 days after nasal instillation. The number refers to the sum of the mathematical values used to denote the presence and severity of symptoms: 0 indicates none; 1, mild; 2, moderate; 3, severe.

† Days after challenge before the appearance of a sharp increase in symptoms.

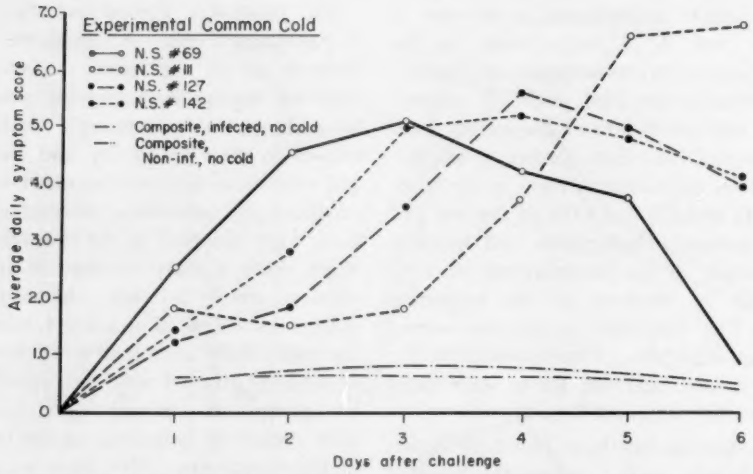

Fig. 8.—The time relationship of symptoms among volunteers who developed a cold after the nasal instillation of one of four infectious secretions.

produced among volunteers. A comparison of the four secretions is shown in Figure 8. The data suggest that the incubation periods were less than 24 hours for No. 69 and 24, 48, and 72 hours, respectively, for the others. For each secretion the peak of symptoms came approximately 48 hours after the end of the lag or incubation period. The course of the illness among males and females was generally parallel.

Comment

At the outset of the discussion one may inquire, "What is a common cold?" Although several investigators as well as we have succeeded in producing symptoms referable to the upper respiratory tract by the instillation of filtered nasal secretions,[3-8] the objection might be raised that these symptoms were merely reactions to the foreign material that was introduced. We do not believe this objection is valid because (1) the donors' secretions had been diluted 100 times with an isotonic solution before they were instilled; (2) foreign protein was added to both the infectious and the control solutions in the form of Group O hemoglobin or yeast extract; (3) some nasal secretions also obtained from patients with symptoms of an upper respiratory infection and presumably containing similar amounts of protein failed to cause any more symptoms than control solutions, and (4) the symptoms of an experimentally induced cold did not begin immediately after instillation of an infectious secretion but after a latent interval of 24 to 72 hours.

If it is accepted that infections were transmitted from the donor secretion to the experimental recipient, can the disease be diagnosed with certainty? While subclinical infections undoubtedly occur and while a more objective test would be desirable, it appears that upon the basis of the criteria we have developed the presence of a cold can be diagnosed with the degree of certainty and uniformity that is necessary for comparative studies. An experimental cold, however, cannot be readily detected by examination of the nose and throat even by a person well qualified in otolaryngology. The great variation in the appearance of the nasopharyngeal mucosa among average well persons is in part responsible for this difficulty in the identification of symptomatic persons by examination.

The regularity with which an experimental cold was produced when a sufficient number of volunteers were challenged and the striking similarity of the illnesses caused by several different secretions that we have used and to the results of previous investigators

suggests that the common cold is a distinct infectious entity. Andrewes [9,10] has noted the preservation of infectivity of the common cold virus after storage at −76 C for at least two years. From our experience it appears that this may be a variable characteristic of infectious nasal secretions, but in most secretions the infectious agent is stable under conditions suitable for the preservation of viruses.

The rate of 10% of persons per week with spontaneous colds which was observed among the noninfected volunteers in our study agrees well with epidemiologic data in other reports.[11-13] The percentage of persons susceptible to an infectious challenge was somewhat less in our studies than the percentage reported by Andrewes and his colleagues.[10,14] This discrepancy probably resulted from the fact that our criteria of a cold were more strict. These were more extreme than would have been necessary if volunteers had been isolated during the preinoculation and follow-up periods, as Andrewes and his co-workers have done. Contrary to the published findings of the English workers [10] and some epidemiologic surveys,[11,13,15] females were not significantly more susceptible than males to an infectious challenge.

The experimental cold among adults was an afebrile illness, the temperature observations presented and the subjective sensation of chilliness in some subjects, however, suggest that a person's thermal regulation might be altered as a result of infection. The nature and severity of the symptoms induced experimentally were typical of spontaneous colds. The nasal instillation of live adenovirus from a tissue culture harvest,[16] live egg-passaged influenza virus,[17] bacteriophage, noninfectious nasal secretions, and several foreign chemicals [18] have not caused symptoms after instillation into the nares. Serologic tests upon the volunteers have consistently failed to show any evidence that the experimental illness was caused by one of the known respiratory viruses or bacteria.

The bacteriologic flora of the nose and throat have received considerable attention at times as a possible cause both of the common cold and of complications.[19,20] The present studies failed to confirm the significance of either of these hypotheses, at least so far as experimentally induced infections were concerned, and also showed no protective action from any particular bacterial flora.

The differences in the incubation or lag period of the illnesses caused by secretions isolated from different persons and in different years is of special interest and may have importance in distinguishing different strains or types of the infectious agent(s). Upon the basis of these studies the incubation period of the common cold might be less than 24 hours or more than 72 hours. Agents that have a relatively long incubation period have also been encountered by the workers at the common cold unit in England.[21] Although other factors could be responsible for the variations in the duration of the incubation periods, they do not appear to be caused by the sex of the volunteers and preliminary studies on the size of the infectious inoculum make it unlikely that this accounted for the difference.

Summary

Observations on more than 1000 volunteers demonstrated that the common cold could be transmitted with regularity by the nasal instillation of dilute, cell-free, nasal secretion from donors with a common cold. Some secretions were stored more than a year without loss of infectivity. Criteria for the recognition of the experimental symptomatic illness have been presented. Experimental colds developed in approximately 35% to 40% of young adults following a single challenge with an infectious secretion. Neither sex, season, nor the source of the different secretions significantly altered susceptibility to an experimental challenge. The incubation period varied from 24 hours or less to 72 hours and was different for secretions collected from different donors and in

different years. Headache, sneezing, chilliness, and sore throat were early symptoms of infection. Nasal discharge, nasal obstruction, cough, and malaise followed the onset by approximately 48 hours and were the severest symptoms. Nasopharyngeal examination was of no value in the identification of infected persons. The experimental cold was rarely associated with fever, and some subjects showed a transient depression of the oral temperature during the early stages of the cold. The bacteriologic flora of the nose and throat neither predisposed to, prevented, nor complicated experimental infections.

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Intrathecal Amethopterin in Neurological Manifestations of Leukemia

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The difficulty in managing acute leukemia in children is enhanced by the occurrence of neurological manifestations. It is especially true when this component of the disease occurs, as it may, during a period with few positive physical findings and a bone marrow in remission. Prior to the use of current chemotherapeutic agents, leukemic-cell invasion of the parenchyma of the brain, meninges, and cranial nerves was noted frequently at postmortem examination.[1,2] At times this involvement was closely correlated with clinical symptoms which presented early in the course of the disease. At present the leukemic child who develops central nervous system manifestations often does so while being given maintenance antileukemic therapy months after the initial diagnosis is made. This suggests that these current agents are only partially effective against the leukemic cell in the central nervous system in the conventional dosage and route of administration. To supplement the incomplete response of current chemotherapy of the otherwise "controlled" acute leukemia, irradiation to the entire brain, cord, or specific region involved can be used with gratifying results initially. However, relapses occur quickly and radiation becomes subsequently less effective even at higher dosages. The failure to control the central nervous system involvement of acute leukemia with conventional chemotherapy and the hestitancy by some radiotherapists to use irradiation for this purpose stimulated a search for a different approach to the problem. It was postulated on the basis of preliminary data [3,15] that the therapeutic agent given orally or parenterally might not gain access to the cerebrospinal fluid or brain parenchyma in the same relative concentrations as in the serum and somatic organs. An experiment was designed to investigate the concentration of the antimetabolite amethopterin (Methotrexate;* 4-amino-N^{10}-methyl pteroylglutamic acid), used because of its solubility, ease of handling, and measurability in the serum, cerebrospinal fluid, and urine after oral and intrathecal instillation.

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Methods and Material

Part I.—Healthy mongrel female dogs were anesthetized with intravenous pentobarbital sodium. An animal, while lying on its side, was restrained so that the back and head were maximally flexed. A smear and total nucleated cell count on iliac marrow were obtained, a French catheter was inserted into the bladder, and blood was collected from a vein in an extremity and allowed to clot. A 20 gauge pediatric length spinal needle was then inserted into the major cistern at the base of the skull by directing the needle slightly caudally in the midline distal to the skull and proximal to the first

* The Lederle Laboratory Division of the American Cyanamid Company supplied this compound in 5 mg. vials for parenteral use.

cervical vertebra. From 0.1 to 1.8 mg. per kilogram of amethopterin diluted with the dog's own cerebrospinal fluid was instilled intrathecally. This was followed by frequent sampling of the cerebrospinal fluid, blood, and urine after brief anesthesia over a five- to six-day period. Samples were routinely stored in a Deepfreeze until a number of specimens were available for determination of amethopterin. Unknown specimens were diluted from 1:10 to 1:1000, and 0.09 ml. was then delivered to paper disks (Schleicher & Schuell penicillin assay disks, 12.7 mm. in diameter), placed on a pour plate of Difco folic acid medium containing 1 mγ/ml. of pteroylglutamic acid and inoculated with Streptococcus faecalis (ATCC 8043) (supplied by Dr. D. J. Hutchison). After the plates were incubated at 37 C for 18 to 24 hours, the zones of inhibition were measured and calculated against standard zones.[4,5]

Part II.—Five children with central nervous system manifestations, four with acute leukemia in various stages of bone marrow involvement, and one with lymphosarcoma with a relatively normal bone marrow were studied. After confirming symptoms by observation in the hospital and substantiating clinical findings, a lumbar puncture was done and the fluid examined for protein, sugar, chloride, and cells. Cultures for routine bacteria and fungus, determination of lactic dehydrogenase activity† (hereafter referred to as LD), and viral tissue culture and mouse inoculation‡ were also done on the cerebrospinal fluid. Two serum samples were sent for viral encephalitides agglutinations. Bone marrow examinations, complete blood cell count, platelet count, and reticulocyte count were done weekly, and hemoglobin and white blood cell count were done daily. All antibiotics and chemotherapeutic drugs were discontinued at least two days prior to the study period. One patient, while taking amethopterin orally 2.5 mg. per day, was given an oral test dose of 10 mg., and the cerebrospinal fluid and serum levels were determined for amethopterin for an 18-hour period thereafter. This served to validate the bioassay technique as currently used and allowed for comparison with amethopterin values of normal adults after test doses.[4] Two children had electroencephalographic studies done prior to and following treatment. Following these evaluations, the children were given 0.1 to 0.5 mg. per kilogram of amethopterin intrathecally through a lumbar puncture at the L4-5 level. Twenty-four-hour urine specimens were collected by voided specimens in four and by catheter in one patient. Frequent blood and cerebrospinal fluid samples were obtained without anesthesia. Determinations of amethopterin levels were made by the bioassay technique. Careful clinical evaluation was carried out during this study period in the hospital and later when seen at weekly intervals in the chemotherapy outpatient clinic.

† Dr. Felix Wroblewski's laboratory.

‡ Dr. Chester Southam's laboratory.

Laboratory Results

Part I.—Figure 1 illustrates the serum and cerebrospinal fluid levels of amethopterin in a dog after an intrathecal dose of 1.8 mg. per kilogram. It is noted that at this dosage initially very high cerebrospinal fluid levels (15,000 mγ per milliliter) were

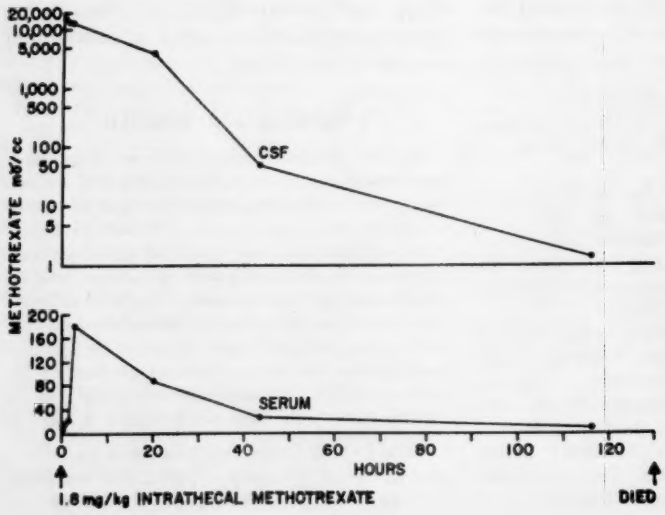

Fig. 1.—Serum and cerebrospinal fluid levels in a dog after intrathecal instillation of 1.8 mg. per kilogram of amethopterin. The dog died at 130 hours.

obtained. In part this was probably due to a puddling of the drug in the cistern before diffusion, since in this experiment the dog was kept lying in one position while anesthetized for three hours with the spinal needle in place. There was a 15-minute delay before amethopterin was measurable in the blood. Both cerebrospinal fluid and serum levels fell at a fairly uniform rate. The cerebrospinal fluid level declined to approximately 4000 mγ per milliliter by 20 hours and to 56 mγ per milliliter by 43 hours, while the serum levels tended to be sustained at a lower level (25 mγ per milliliter). At 116 hours, small, but measurable amounts of amethopterin were detected in cerebrospinal fluid and serum. Clinically, this dog had a progressively downhill course, with weight loss, anorexia, bloody diarrhea, and death in five days, simulating lethal toxicity following oral or intravenous administration of the antifolics, as has been described.[6,7] The bone marrow at 116 hours revealed a total nucleated cell count depression of 210,000 to 40,000 and a definite megaloblastosis. The central nervous system was examined, and no evidence of bleeding or other damage was found. Three other dogs tolerated 0.1 to 0.5 mg. per kilogram of amethopterin intrathecally very well, without weight loss, lethargy or bloody diarrhea; but they did show megaloblastosis in the bone marrow and a decrease in the total nucleated cell count in the marrow.

Part II.—Figure 2 represents the blood and cerebrospinal fluid levels of amethopterin in a 10-year-old child with lymphosarcoma after taking 10 mg. of amethopterin (0.33 mg. per kilogram) orally after fasting. The patient had been given 2.5 mg. daily of oral amethopterin for seven days before the test. Amethopterin appeared in the cerebrospinal fluid one hour after the oral dose and reached the level of only 15 mγ per milliliter in three hours and fell to 2 mγ per milliliter in 18 hours. The serum level rose from 2 mγ per milliliter before administration to a level of 900 mγ per milliliter in 30 minutes and then fell precipitously at three hours and was detected only at 5 mγ per milliliter at 18 hours. It is emphasized that an oral dose four times greater than the amount conventionally used gave a cerebrospinal fluid value of 18 mγ per milliliter of amethopterin 30 minutes later in this patient.

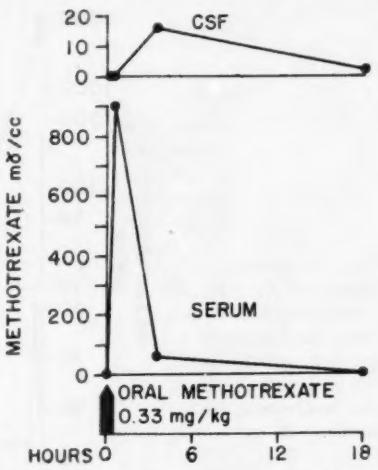

Fig. 2.—Levels in serum and cerebrospinal fluid after oral administration of 10 mg. (0.33 mg. per kilogram) of amethopterin after fasting in a 9-year-old boy with lymphosarcoma and central nervous system involvement.

Figure 3 depicts the cerebrospinal fluid and serum levels of amethopterin after intrathecal therapy in a semicomatose leukemic child whose bone marrow was in remission. The cerebrospinal fluid level at one hour after injection was 5000 mγ per milliliter, while the serum level was only 40 mγ/ml. Cerebrospinal fluid values fell to 475 mγ per milliliter at 18 hours and to 40 mγ per milliliter at 43 hours after a dose of 0.1 mg. per kilogram, and serum levels were not measurable at this time. At the 0.5 mg. per kilogram dose, cerebrospinal fluid levels of 430 mγ per milliliter and serum levels of 17 mγ per cubic centimeter of amethopterin were found at 48 hours.

Another child with moderate neurological involvement and with a bone marrow in relapse was given intrathecal therapy, and the cerebrospinal fluid values are plotted on Figure 4. Elevated protein, white cell count

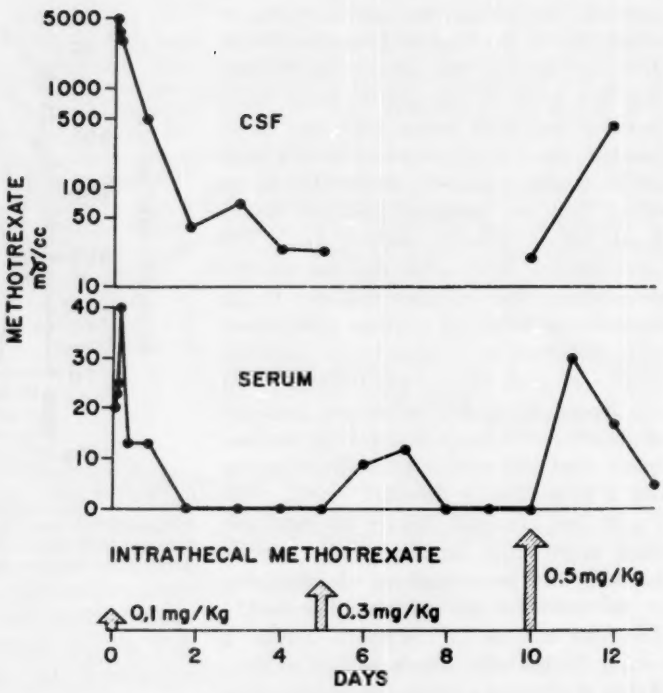

Fig. 3.—Serum and cerebrospinal fluid levels of amethopterin after intrathecal instillation in a 5-year-old girl with severe central nervous system involvement due to leukemia.

(up to 8000 per cubic millimeter) and LD, with a very low glucose value (2 mg.) per 100 cc. when the white blood cell count was 8000) and normal chloride determinations,

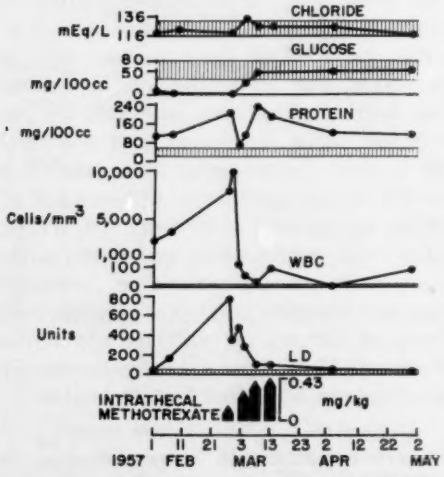

Fig. 4.—Cerebrospinal fluid determinations of chloride, glucose, protein, white blood cells, and LD in a 9-year-old boy with moderate neurological manifestations of acute leukemia.

preceded intrathecal amethopterin therapy. There followed a precipitous and sustained fall in the leukocyte count and LD levels; however, after an initial fall, the protein level rebounded and then fell slowly but did not reach normal range.

Cerebrospinal fluid levels of amethopterin in five patients with neurological involvement are depicted in the scattergram in Figure 5. At 15 minutes the cerebrospinal fluid levels were very high (approximately 5000 mγ per milliliter), but they fell gradually at 48 hours to levels of 20 to 500 mγ per milliliter and at 96 hours to levels of 2 to 100 mγ per milliliter. In three cases measurable amounts were found in the cerebrospinal fluid six days after intrathecal administration of the drug. The corresponding serum levels in the same patients ranged from 20 to 60 mγ/ml. at 15 minutes and fell less rapidly than the cerebrospinal fluid levels, so that amounts of 2 to 21 mγ per milliliter were noted frequently at 48 hours, and at five days levels of up to

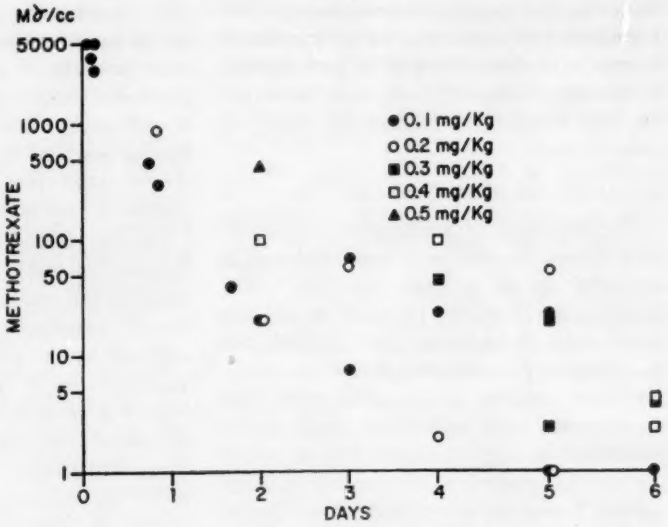

Fig. 5.—Scattergram of cerebrospinal fluid levels of five patients with neurological involvement of leukemia or lymphosarcoma after intrathecal amethopterin instillation.

6 my per milliliter were seen occasionally in patients receiving the higher doses.

From 14% to 33% of the intrathecal dose was excreted in the urine in a four- to six-day period. About 85% of this amount is recovered in the first 48 hours.

Clinical Results

All five patients treated with intrathecally administered amethopterin improved clinically. One youngster, who was semicomatose, hydrocephalic, and unable to see (but who could chew, swallow, cry, and say "Mama") prior to intrathecal therapy, 14 days after therapy was able to read, make sentences, carry on a conversation, and play games which required thought processes. This patient also had spastic paralysis of the lower extremities and a flaccid paralysis of the upper extremities. Eighteen days after therapy she had moderate use of both arms and hands and the spasticity in the lower extremities abated to a large measure. This remission in symptoms, which began about 10 to 14 days after initial intrathecal therapy, lasted about 6 weeks.

Three boys with symptoms of headache, lethargy, nausea, and vomiting and with findings of papilledema and exhibiting various stages of bone marrow involvement improved markedly after intrathecal therapy. About four to seven days were required for symptoms to start abating. Positive effects lasted for four to six weeks. Papilledema was reduced or absent in 7 to 14 days. Four of the five patients treated revealed megaloblastosis in the bone marrow, but there was no change in the over-all course of the disease or influence on the peripheral blood picture.

One youngster with lymphosarcoma involving the mediastinum and testis, as well as the central nervous system, was lethargic, apathetic, withdrawn, and bed-ridden due to weakness in the upper and lower extremities. He regained the use of his limbs, was able to walk, and again exhibited his former personality about two weeks after therapy. A similar neurological picture had been seen previously and had responded to intravenous mechlorethamine hydrochloride (nitrogen mustard) for a period of three to four weeks. At the onset of a second such episode, irradiation was given to the head and spinal cord with essentially no response for a three-week period. It was at this time that intrathecal therapy was given. It is quite possible that the clinical effect noted could be due in part to, or synergistic with,

the x-ray therapy. Electroencephalographic tracings in two patients prior to intrathecal therapy were determined to be pathological. Follow-up tracings in both were improved but still revealed an underlying abnormal pattern.

Comment

The frequency of neurological manifestations during the course of acute leukemia is estimated to be as high as 50%.[8] The incidence at Memorial Hospital in children would seem to be nearer 10% to 20%, but this frequency is sufficient impetus to search for more efficient means of therapy. The experiments with intrathecal amethopterin instillation in dogs indicated that up to 0.5 mg. per kilogram is well tolerated although causing a megaloblastosis and a mild depression of the total nucleated cell count in the marrow. A dose of 1.8 mg. per kilogram in one dog was lethal, presumably due to the prolonged serum levels which produced systemic toxicity similar to that seen after oral administration of frequent large doses of amethopterin. This is in line with the findings of Philips and Thiersch that the toxicity of a given total dose of amethopterin is enhanced tenfold by frequent administration as contrasted to a single dose. It would appear that amethopterin from the spinal depot is released slowly into the blood to give these prolonged levels. A search of the literature reveals one mention of intrathecal therapy in acute leukemia with use of aminopterin (0.025 mg. for two days and 0.036 mg. for six days).[9] With this dose, a reduction of cerebrospinal fluid leukocyte count was noted without obvious systemic effect.

In children with neurological complications, 0.5 mg. per kilogram proved to be well tolerated in the manner given without signs of meningeal irritation. Clinical symptoms rapidly improved at this dose level in all patients. There was no alteration of the peripheral blood picture due to this therapy. Megaloblastosis was noted 7 to 10 days after instillation of amethopterin. In two patients with bone marrows revealing over 50% megaloblasts, the relapse was not affected by this dosage even when repeated three times in 10 days. One child had been given oral amethopterin and was thought to be refractory, and another child was relapsing while given the drug. Nevertheless, clinical improvement of the neurological manifestations occurred.

The development of only a threefold to fivefold resistance to amethopterin by the leukemic cell would be sufficient to make the disease completely refractory to any dose tolerated by the oral, intravenous, or intramuscular route. As was demonstrated in Figure 3, however, the levels developed in the spinal fluid after intrathecal dosage are 30 to 100 times as high as those obtained in the cerebrospinal fluid after even a massive single oral dose. Thus it would seem reasonable to suppose that even in patients whose systemic disease had become refractory to amethopterin, the intrathecal use of amethopterin would produce improvement in the signs and symptoms due to meningeal involvement.

There was no demonstrable evidence that the neurological manifestations in these patients was due to bacterial, fungal, or viral infection. All blood and cerebrospinal fluid cultures failed to grow bacteria or fungi. Cerebrospinal fluids were inoculated into mouse brain and tissue culture without showing evidence of the presence of a virus. Serum agglutination titers failed to reveal evidence of any of the known types of encephalitis. From these studies infections with viruses of Eastern and Western equine, St. Louis, or Japanese B encephalitis groups, herpes simplex, influenza, poliomyelitis, and Newcastle disease can be ruled out.

It is presumed that the neurological manifestations exhibited by these patients were due to leukemic involvement of the brain, meninges, and/or cranial nerves, as has been described.[8-14] None of these children have died, so that exact localization and type of lesions responsible for symptoms must remain speculative. From the lack of clinical signs, it is evident that the intrathecal

amethopterin in the dosage used did not cause a detectable chemical irritation of the meninges.

Summary

Dogs tolerated up to 0.5 mg. per kilogram of amethopterin (Methotrexate) intrathecally and yet exhibited megaloblastosis and depression in the nucleated-cell count in the bone marrow. A single dose of 1.8 mg. per kilogram intrathecally caused death from marrow depression and damage to gastrointestinal epithelium.

Five patients with neurological symptoms due to leukemia and lymphosarcoma improved markedly on 0.1 to 0.5 mg. per kilogram intrathecally. The neurological remissions began about one week after instillation and lasted about six weeks. Repeated neurological remissions were obtained in all patients.

There was improvement in the electroencephalograph tracings in two patients after amethopterin intrathecally.

Intrathecal amethopterin produced a neurological remission in two patients who were considered refractory to the drug orally.

Intrathecal instillation yielded extremely high cerebrospinal fluid levels and prolonged serum levels of amethopterin.

In these patients, intrathecal therapy produced no change in the peripheral blood picture and did not significantly alter the differential count in the bone marrow other than producing megaloblastosis.

Amethopterin was given to patients in repeated doses with no ill effects. It would appear that such intrathecal injection may well become a useful tool in the armamentarium of the clinician treating acute leukemia.

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Binding of Corticosteroids by Plasma Proteins

V. Corticosteroid-Binding Globulin Activity in Normal Human Beings and in Certain Disease States

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The role of the blood plasma in the transport of nutrients, metabolic intermediates, and hormonal agents has long been recognized, yet there is now an increasing awareness of the degree to which plasma proteins may be specialized for this function. Uniquely adapted proteins are responsible for the transport of nearly all the neutral fat, cholesterol, and phospholipids of the plasma in colloidal solution.[1] The free fatty acids of plasma, which have recently been shown to be of great importance in fat transport from the peripheral depots to the sites of oxidation, circulate nearly completely bound to the plasma albumin.[2] Albumin also binds many other substances of physiologic significance. Most of the circulating iron of the plasma is bound to an iron-binding globulin.[3] The degree of binding of thyroxine to a thyroxine-binding protein is well known and is made the basis of the familiar clinical index of thyroid function, the protein-bound iodine. The thyroxine-binding globulin migrates electrophoretically at pH 8.8 intermediate between the α_1- and α_2-globulin regions.[4] A globulin of somewhat similar properties has a specific affinity for free hemoglobin, with which it appears to unite irreversibly.[5] It is an α-globulin again which is responsible for most of the binding of circulating cyanocobalamin (vitamin B_{12}) [6].

It has been reported previously from this laboratory that at physiologic concentrations corticosteroid hormones circulate in the plasma nearly completely bound to protein.[7] At least two corticosteroid-binding proteins exist in human plasma. The ability of serum albumin to bind corticosteroids has been clearly established.[8,9] Albumin binds hydrocortisone relatively weakly but less polar steroids more strongly. The corticosteroid-binding ability of albumin is not saturated at physiologically attainable steroid levels because of the large amounts of albumin present.

Evidence for a second corticosteroid-binding protein has been obtained using C^{14}-labeled hydrocortisone and corticosterone.[10] When small amounts of the radioactive corticosteroid were added to normal human plasma in a cellophane bag and dialyzed against a buffer of physiologic pH, nearly all the hormone was found to be present within the plasma. With larger additions of the corticosteroid under study, the amount of steroid which was found within the buffer rose progressively. Three significant properties of corticosteroid binding indicated that serum albumin could not be responsible for the degree of binding observed. First, the degree of binding exceeded that which could be observed with purified human albumin preparations. Second, whereas albumin binding is saturated with steroid only at high steroid concentrations, the binding of hydrocortisone by human plasma decreased remarkedly with only small additions of hydrocortisone. Third, the ability of related steroids to displace radioactive hydrocortisone from the plasma protein of high binding affinity indicated that this protein bound hydrocortisone more firmly and less polar steroids more weakly. Chemical alterations in the hydrocortisone molecule often of a minor nature greatly reduced the affinity of

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Dr. France Alexander assisted in clinical studies and Mrs. Ida Kozak made the radiochemical measurements.

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the derived steroid for the hydrocortisone-binding site.

On the basis of this evidence the existence of a relatively specific corticosteroid-binding protein was predicted. Subsequently, using continuous flow electrophoresis, binding of hydrocortisone-4-C^{14} and corticosterone-4-C^{14} by an α-globulin has been demonstrated.[11] This protein, which has been named "corticosteroid-binding globulin," appears to have the electrophoretic properties of a glycoprotein in that it moves anodally at pH 5.2.

In this paper observations will be presented concerning the corticosteroid-binding activity of normal human plasma and the plasma from patients with a variety of diseases. Preliminary observations will also be presented which show that the pattern of corticosteroid binding differs greatly in man and in the dog.

Methods

The conditions of dialysis, analytical methods, and techniques of counting radioactive steroids have been described elsewhere.[10] In brief, 10 ml. of heparinized plasma were pipetted into a cellophane bag, and the bag was placed into a large test tube containing 80 ml. of Krebs phosphosaline buffer, pH 7.4, to which had been added 0.5 mcg. of hydrocortisone-4-C^{14}. In one experiment different amounts of hydrocortisone were added to samples of human, dog, and rat plasma. In certain experiments where the amount of plasma available was insufficient, 5 ml. of plasma was dialyzed against 40 ml. of buffer.

After stoppering, the test tube was placed on a rotating wheel for 48 hours at 4 C to allow equilibration of the steroid between plasma and buffer. The concentrations of radioactivity within the buffer and plasma were then measured as well as the protein concentration of the plasma.

The results have been presented in two ways. First, the percentage of unbound steroid within the bag has been calculated from the concentration of radioactivity in the buffer and the total radioactivity inside the bag. Second, an index of affinity has been calculated as a means of comparing the affinity of the plasma for hydrocortisone per milligram of protein under the specified conditions tested. The value of this index is obtained from the following formula:

$$C = \frac{\text{Bound hydrocortisone}}{\text{Free hydrocortisone}} \quad \frac{1}{\text{Protein conc. (milligrams per milliliter)}}$$

TABLE 1.—*Corticosteroid-Binding Globulin Activity in Plasma from Normal Adult Human Beings*

Case	Sex	Age, Yr.	C *	Unbound F, %
1	M	39	2.1	0.8
2	M	24	1.3	1.3
3	M	25	1.0	1.4
4	M	30	1.3	1.2
5	M	36	1.1	1.5
6	M	26	2.2	0.7
7	F	39	1.6	1.0
8	F	28	1.6	0.9
		Mean	1.52	1.10

* Index of affinity.

It should be emphasized that this value is not a constant but is dependent of the steroid concentration.

Human plasma was obtained two to three hours after breakfast. Rat plasma was obtained from adult male rats of the Sprague Dawley strain. The dog plasma was from an adult mongrel dog in apparent good health.

Results

The plasma from normal adult humans was able to bind nearly all the hydrocortisone added to the dialysis system (Table 1). Within the dialysis bag nearly 99% of the hydrocortisone was protein bound. Considerable uniformity is evident in this small sample of normal adults.

It was of interest to examine the plasma of pregnant women for evidence of increased corticosteroid-binding globulin activity. Such a possibility was suggested by the finding of Dowling, Freinkel, and Ingbar[12] that thyroxine-binding globulin activity is increased during pregnancy and that the elevations of the plasma protein-bound iodine in pregnancy could be attributed to this alteration. The plasma 17-hydroxycorticosteroids have likewise been found to be elevated in pregnancy.[13] The experimental results (Table 2) do not suggest any in-

TABLE 2.—*Corticosteroid-Binding Globulin Activity in Plasma of Pregnant Women*

Case	Age, Yr.	Duration of Pregnancy, Mo.	C *	Unbound F, %
9	22	4½	0.7	2.2
10	16	5	0.5	2.9
11	20	5	1.0	1.5
12	24	6	1.0	1.5
13	24	9	0.7	2.2

* Index of affinity.

TABLE 3.—*Corticosteroid-Binding Globulin in Plasma of Patients with Gross Abnormalities of Plasma Globulin*

Case	Sex	Age, Yr.	Diagnosis	C *	Unbound F, %
14	F	56	Xanthomatous biliary cirrhosis	0.54	2.2
15	M	..	Portal cirrhosis	0.22	6.1
16	F	56	Portal cirrhosis	0.40	3.9
17	F	51	Acute hepatic necrosis	0.07	15.9
18	F	25	Nephrotic stage of glomerulonephritis	0.33	6.2
19	M	53	Multiple myeloma	0.19	5.3
20	F	68	Sarcoidosis (?)	0.24	7.1
21	M	32	Chronic lymphedema, (diffuse hyperglobulinemia)	0.40	4.9

* Index of affinity.

creased corticosteroid-binding activity in pregnancy. The unbound steroid in plasma from pregnant women was found to be slightly higher than in the normal controls, but this finding is difficult to assess because of the increased concentration of 17-hydroxycorticosteroids and other steroids of the plasma in this state which might compete with the labeled steroid for the corticosteroid binding sites.

Remarkable abnormalities in the corticosteroid-binding globulin activity were found in patients with gross plasma protein abnormalities of several types (Table 3). There was, however, little increase in the unbound hydrocortisone in a patient with xanthomatous biliary cirrhosis with a very high plasma cholesterol. The plasma transport of cholesterol and hydrocortisone are quite unrelated. Severe liver disease in the few patients examined is often accompanied by a decrease in the corticosteroid-binding globulin activity. In Case 17, with acute hepatic necrosis, the degree of binding is notably less than normal. Lower deficiencies of corticosteroid-binding globulin activity were observed in the other patients.

TABLE 5.—*Corticosteroid-Binding Globulin Activity in Patients with Miscellaneous Diseases*

Case	Sex	Age, Yr.	Diagnosis	C *	Unbound F, %
27	F	44	Exfoliative dermatitis (prolonged cortisone treatment)	0.55	3.5
28	M	47	Subacute bacterial endocarditis	1.30	1.2
29	F	31	Acute pyelonephritis (convalescent)	1.06	1.2
30	F	55	Thrombophlebitis, cholecystitis	1.33	1.1
31	F	85	Myocardial infarction	0.89	1.8
32	F	78	Pulmonary tuberculosis	0.89	1.6
33	F	69	Hodgkin's disease	0.71	2.2

* Index of affinity.

TABLE 4.—*Corticosteroid-Binding Globulin Activity in Patients with Endocrine Diseases*

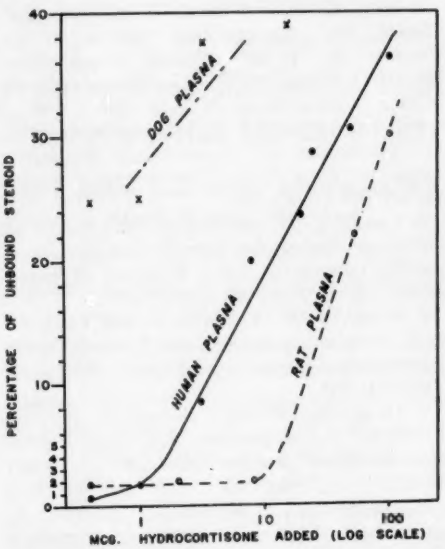
Case	Sex	Age, Yr.	Diagnosis	C *	Unbound F, %
22	F	24	Hyperthyroidism (2 mo. pregnant)	0.56	2.4
23	M	68	Addison's disease	0.99	1.5
24	M	25	Hypopituitary dwarf	0.85	1.8
25	F	39	Acromegaly	1.72	0.8
26	F	64	Myxedema	0.90	2.1

* Index of affinity.

No suggestive alterations in binding activity were found in an exploratory survey of patients with endocrine diseases (Table 4). The man with Addison's disease had been given intermittent treatment with corticosteroids for many years.

A number of plasmas from patients selected at random on the wards were examined (Table 5). Case 27 was a woman with exfoliative dermatitis who had received protracted corticosteroid treatment prior to obtaining the sample. The increased amount of unbound hydrocortisone found after dialysis could be a result of either the primary disease or its treatment. Cases 28 to 32 were patients convalescent or only moderately ill at the time the plasma was obtained. Two of these patients were selected because of their advanced age. Significant abnormalities of corticosteroid-binding globulin were not observed in this group.

The corticosteroid-binding globulin activity of human plasma has been compared to that from the dog and the rat (Figure). In these experiments the degree of binding was examined with increasing amounts of hydrocortisone added to the dialyses. The type of curve obtained with human plasma demonstrates the saturation of the corticosteroid-binding globulin activity with small additions of hydrocortisone. At low concentrations of steroid the binding is almost exclusively limited to the corticosteroid-binding globulin, but albumin binds an increasing portion of hydrocortisone as the amount of the hormone added to the system rises. It would appear that the capacity of rat plasma



The percentage of unbound hydrocortisone in plasma is plotted against the amount of hydrocortisone added to a standard dialysis equilibrium system consisting of 10 ml. of plasma and 80 ml. of buffer. Experiments with human, dog, and rat plasma are compared.

to bind added hydrocortisone exceeds that of human plasma. Conversely, little evidence was obtained indicating corticosteroid-binding-globulin activity in dog plasma. Most of the binding which occurred could have been due to binding by albumin. This study is now being extended to other species.

Comment

The ability to bind nearly all the hydrocortisone present in plasma at physiologic concentrations appears to be a consistent property of plasma from normal adults. Deficiencies of this binding ability are apparently common in a number of diseases with gross disturbances of plasma proteins. The extreme lowering of the corticosteroid-binding globulin activity in a patient with acute hepatic necrosis suggests that this protein is of hepatic origin as are so many of the plasma proteins.

The inability, in this preliminary survey, to detect clinical conditions associated with greater than normal corticosteroid binding deserves comment. Although this condition may not occur, the experimental design was not ideal to detect it. As was noted with rat serum, increased binding capacity might become obvious only with dialyses carried out with larger hydrocortisone additions to the system than used routinely in this study.

The physiologic implications of corticosteroid binding by plasma are not entirely clear. The ability of plasma to bind hydrocortisone accounts for the virtual absence of the active hormone from the urine of normal human beings.[7] A puzzling condition where the prevention of renal excretion might be disturbed is the prolonged excretion of active corticosteroids in the urine as measured by the glycogenic assay after surgical or other trauma.[14] Recent measurements of blood levels of 17-hydroxycorticosteroids have failed to establish an increase in plasma levels of such a duration.[15] A possible explanation of this finding would be that corticosteroid-binding globulin activity is decreased during the period of the prolonged urinary excretion of glycogenic corticoids.

The degree of protein binding which has been demonstrated must impose a major restraint on the passage of hydrocortisone through the capillary membranes. Transfer probably occurs by way of the unbound component. The eventual concentration of steroid in the interstitial fluid depends on the rate of transcapillary transfer, the corticosteroid-binding ability of the fluid in question, and the affinity of the cells for corticosteroids. The high hepatic clearance of active corticosteroids indicates an unusual affinity for corticosteroids or an actual trapping mechanism within the hepatic cells.

Certain significant questions arise from these observations about the interpretation of the "free" 17-hydroxycorticosteroid levels of the plasma. This term denotes that fraction of the total 17-hydroxycorticosteroids which are not conjugated with glucuronic acid. The physically unbound hydrocortisone of plasma represents only a small fraction of this moiety. Are the physiologic effects of the corticosteroids determined by the unbound or total steroid level of the plasma? Robbins and Rall [16]

have recently suggested that the physiologic regulation of the thyroid by the pituitary is determined by the minute concentration of the unbound form of the hormone.

A teleologic interpretation of the presence of a specialized corticosteroid-binding protein is difficult. Because these proteins impede the transfer of steroid across the capillary membrane from the blood stream to the cells, the term "transport protein" is inappropriate. The action of corticosteroid-binding globulin to inhibit the renal excretion of active corticosteroids is not a satisfying explanation of its real function. More likely, the presence of the relatively greater concentration of bound steroid stabilizes the physiologically significant concentration of unbound steroid.

Summary

The binding of hydrocortisone at physiologic concentrations by human plasma has been measured after equilibrium dialysis with use of hydrocortisone-4-C^{14}. Hydrocortisone was nearly 99% bound to the plasma of eight normal human beings. This high degree of binding can be attributed to a relatively specific corticosteroid binding globulin.

No evidence of increased corticosteroid binding was found in plasma from five pregnant women.

Impaired binding of hydrocortisone by plasma was observed in severe liver disease and several other conditions associated with gross abnormalities of the plasma proteins.

Little corticosteroid binding activity was found in dog plasma as compared to human plasma, whereas rat plasma appeared to have a higher corticosteroid-binding capacity than human plasma.

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Amino Acid Mixtures in the Treatment of Experimental Dietary Cirrhosis in the Rat

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It has been demonstrated that cirrhosis of the liver can be produced regularly in the growing rat by diets low in protein and also deficient in choline or methionine.[1-5] The addition of choline or methionine to these experimental diets retards the development of the disease. However, once the disease is fully established the addition of these lipotropic factors (choline or methionine) to the low-casein diet fails to bring about adequate repair and regeneration of the liver.[5,6] In earlier studies[7] it was shown that after producing cirrhosis in rats with a diet containing 4% casein, supplementation of this diet with relatively high concentrations of choline or methionine for four months induced partial clearing of fat from the cirrhotic livers and caused the death rate to decrease but failed to promote repair and regeneration of the liver. The animals remained stunted and feeble. By contrast, in rats fed a diet containing 30% casein but isocaloric with the 4% casein diet there was almost complete restoration to normal appearing liver cells, no fatty infiltration, and return of normal growth and vigor. Connective tissue in the liver appeared to have decreased, but it was not determinable whether this was a real or apparent change due to hypertrophy of the liver. It was evident, then, that casein contained properties or substances in addition to methionine which fostered recovery from nutritional cirrhosis.

The present study was designed to answer several further questions concerning the effects of dietary factors in the repair and regeneration of the cirrhotic liver. Is the effectiveness of casein due to its content of amino acids? Are the histological changes in fat and connective tissue substantiated by chemical analysis of liver tissue? Cirrhosis was produced by feeding rats a standard 4% casein diet for 16 weeks. As therapeutic agents amino acid mixtures were then substituted for dietary casein. An amino acid mixture as close to the composition of casein as practicable was fed at a 30% level to learn whether the efficacy of casein is related to its amino acid content or to other unidentified substances. All components of this casein-like mixture were kept constant except for DL-methionine, which was fed at three concentrations, namely, 0.14%, 1.05%, and 1.83%. These were calculated to correspond to diets containing, respectively, 4%, 30%, and 50% casein. By varying this one component it should be possible to determine whether methionine exerts a therapeutic effect apart from its role as an essential amino acid. In one subgroup choline was substituted for methionine in order to compare their relative effectiveness in promoting hepatic repair and regeneration.

In previous studies[7] recovery from cirrhosis was indicated by loss of fat, restoration of normal structure, and apparent decrease of connective tissue on microscopic examination of the liver. In order to obtain

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TABLE 1.—*Composition of Experimental Diets*

	Low-Protein, Gm./100 Cc.	High-Protein, Gm./100 Cc.	Amino Acid Mixture (Basal)
Vitamin-free casein	4.0	30.0	--
L-cystine	0.5	0.5	0.62
Cottonseed oil	5.0	5.0	5.0
Salt mixture *	3.0	3.0	3.0
Corn starch	86.5	52.5	52.5
Vitamin mixture †	1.0	1.0	1.0
Amino acid mixture ‡	--	--	36.5
Alphacel	--	8.0	--
Methionine	--	--	0-1.83

* Hubbell, R. B.; Mendel, L. B., and Wakeman, A. J.: J. Nutrition 14:273, 1937.

† Vitaminized cornstarch of which 1 gm. supplied the following in milligrams; thiamine HCl, 1.25; riboflavin, 0.625; calcium pantothenate, 0.625; pyridoxine HCl, 0.25; nicotinic acid, 12.5; para-aminobenzoic acid, 10.0; folic acid, 0.05, 2-methyl-1, 4-naphthoquinone, 0.1 mg; α-tocopherol, 4.0 mg; vitamin A (percomorph liver oil), 750 U. S. P. units, and vitamin D, 108 U. S. P. units, were added per 100 gm. diet in 200 mg. cottonseed oil.

‡ The amino acid mixture was prepared to contain the following in milligrams: L-alanine, 0.99; L-arginine, HCl 1.26; DL-aspartic acid, 4.26; L-cystine, 0.62; L-glutamic acid, 6.975; glycine, 0.63; L-histidine HCl, 0.96; DL-isoleucine, 4.56; L-leucine (methionine-free), 3.03; L-lysine, 2.58; DL-phenylalanine, 1.92; DL-threonine, 2.76; L-tryptophan, 0.39; L-tyrosine, 1.95; DL-valine, 4.62.

a more precise measure of these changes all rat livers at the end of the present experiments were analyzed chemically for their content of fat and collagen.

Methods

Male Albino rats of the Sprague-Dawley strain, 28 days old and weighing between 45 and 55 gm., were employed. The animals were housed in individual cages. The body weights of all animals were recorded at weekly intervals during the entire program (Weeks 1 to 32).

The composition of three diets employed is shown in Table 1. The cirrhosis-producing diet containing 4% casein was patterned after that devised by Daft, Sebrell, and Lillie.[32] In order to prevent kidney damage, to which choline-deficient weanling rats are prone,[8] 16 mg. choline chloride per 100 cc. was incorporated in this diet for the first two weeks of the experiment.

The 30% casein diet previously was shown to promote optimal repair of the liver. This diet was employed as a norm for comparison of therapeutic effects.

An amino acid mixture simulating 30% casein (but free of methionine) was prepared according to data obtained from Block and Bolling[9] and the report on the "Cooperative Determination of the Amino Acid Content and Nutritive Value of Six Selected Food Sources."[10]

In order to compensate for the presence of the poorly utilizable D- optical isomers of threonine, valine, isoleucine, and aspartic acid present in this amino acid mixture,[11-14] nonnutritive cellulose (Alphacel) was incorporated into the 30% casein diet.

TABLE 2.—*Distribution of Animals During Foreperiod of Sixteen Weeks*

	Group No.	No. Animals	Protein Source
	II *	171	4% casein (contains 0.14% methionine)
Normal control	I	5	30% casein (contains 1.05% methionine)
Normal control	III	10	Amino acid mixture (1.05% methionine)

* Given cirrhosis-producing diet.

Methods

1. *Foreperiod.*—One hundred seventy-one rats were fed the 4% casein cirrhosis-producing diet for 16 weeks (Table 2, Group II). As normal controls, 5 animals were fed a 30% casein diet (Table 2, Group I) and 10 animals were fed an amino acid ration containing 1.05% DL-methionine, which simulated a 30% casein diet (Table 2, Group III).

2. *Biopsy Period.*—At the end of 16 weeks, 18 animals were killed for chemical analyses of lipids and collagen in the livers. The remaining animals, both normal controls and cirrhotic, were subjected to liver biopsy under ether anesthesia. Animals in control Groups I and III were allowed to continue on their respective dietary regimens. Animals given the cirrhosis-producing diet (Experimental Group II) were divided into six comparable groups (23 rats each) for testing with various therapeutic diets. However, those animals which exhibited no cirrhosis or only minimal changes on histological section were eliminated. The final grouping of animals under study is shown in Table 3.

TABLE 3.—*Distribution of Animals During Afterperiod of Sixteen Weeks* *

Group		No. Rats Given Diet	Protein Source
Experimental Group II	II G liver biopsy	15	Amino acid mixture; no methionine
	II D liver biopsy	15	Amino acid mixture + 0.14% methionine
	II B liver biopsy	18	Amino acid mixture + 1.05% methionine
	II C liver biopsy	15	30% casein (contains 1.05% methionine
	II E liver biopsy	21	Amino acid mixture + 1.83% methionine
	II F liver biopsy	19	Amino acid mixture + 0.14% methionine + 0.50% choline
Control Groups	II H killed †	18	4% casein (contains 0.14% methionine)
	II A liver biopsy	6	4% casein (contains 0.14% methionine)
	I liver biopsy	5	30% casein (contains 1.05% methionine)
	III liver biopsy	10	Amino acid mixture + 1.05% methionine

* Various "therapeutic" diets were fed the experimental groups.

† This control group was killed at the beginning of this period.

3. *Afterperiod.*—Six groups of animals were fed different therapeutic diets for 16 weeks as outlined in Experimental Group II, Table 3. Three control groups were employed also; these were fed a 4% casein ration, a 30% casein ration, and a 30% amino acid ration containing 1.05% DL-methionine.

Measurements of food intake of the various groups during the second week of the afterperiod were made. It was then possible to limit (with the exception of Group II G, Table 3, which exhibited severe anorexia) animals of all groups to equal food intake during the afterperiod.

After a period of 16 weeks of feeding the respective diets, the experimental program was terminated. Rats of all groups were weighed and then killed by exsanguination with use of light ether anesthesia. The liver and spleen of each rat were rapidly removed. Sections of liver were removed for histopathologic examination. The remaining liver was weighed, wrapped in heavy aluminum foil, frozen in liquid nitrogen, and stored at —30 C until used for quantitative determination of total liver collagen and total liver lipids.

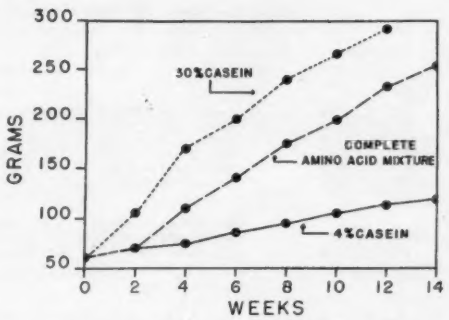
Specimens of tissue were fixed in Zenker's solution and in 10% formalin. The tissue sections were embedded in paraffin by the usual methods, cut at 5μ, and routinely stained with hematoxylin and eosin. Trichrome stains were utilized for the better definition of connective tissue. Acid-fast stains were used for the demonstration of ceroid pigment, and frozen sections were stained with oil red O and hematoxylin for definition of fat.

The quantity of collagen present in the liver was used as a chemical estimate of the degree of fibrosis (connective tissue).[15] The method of Lowry, Gilligan, and Katersky[16] for determining collagen was employed.

The tissue was ground and extracted in a Soxhlet apparatus with a mixture of chloroform and methanol. The total lipids were determined gravimetrically upon the petroleum-ether-soluble fraction of this extract.

Results

1. *Foreperiod.*—The animals fed the 4% casein diet (Group II, Table 2) grew slowly in contrast to the rapid growth of those given the 30% casein diet. During the first two weeks of the foreperiod the animals fed the amino acid mixture simulating 30% casein (Group III, Table 2) exhibited loss of appetite and slight growth. However, by the end of the third week of this period a marked improvement in appetite and growth rate was noted. By the end of the fourth week all animals were consuming approximately the same amount of food, an average of 9 gm. daily.



Average growth curves of animals during 16 weeks of foreperiod.

The Figure shows the average growth curves during this period.

Mortality during the 16-week foreperiod was low. Only two deaths, occurring late in the foreperiod, were noted in Group II (Table 2), which received the 4% casein diet.

2. *Biopsy Interval.*—Ten out of one hundred sixty-three rats subjected to biopsy of the liver died within a week of this procedure. The fatalities usually occurred in the animals exhibiting severe hepatic damage as evidenced by nodularity of the livers and by the accumulation of ascitic fluid.

Gross alteration of the livers, ranging from congestion and enlargement in the early stages of cirrhosis to pallor, granularity, and nodularity with more advanced lesions, was observed at the time of biopsy in 143 out of the 163 rats fed the 4% casein diet.

Microscopically, the huge fatty cysts described by Hartroft[17] seemed to replace up to 90% of the parenchyma. Connective tissue proliferation was evident about the hepatic vein areas and extended in strands along the sinusoids to connect with similar strands developing from the adjacent central zones. In accord with the observations of Himsworth,[18] the connective tissue fibers

TABLE 4.—*Body Weight Change After Sixteen Weeks of "Therapy"*

	Group No.	Ration	Mean Body Weight		% Mean Gain
			Biopsy	Autopsy	
Experimentals	II G	Amino acid mixture; no methionine	131	92	−29
	II D	Amino acid mixture + 0.14% methionine	153	194	27
	II B	Amino acid mixture + 1.05% methionine	133	227	70
	II C	30% casein (contains 1.05% methionine)	134	259	92
	II E	Amino acid mixture + 1.83% methionine	150	233	56
	II F	Amino acid mixture + 0.14% methionine + 0.50% choline	142	205	45
Controls	I	30% casein (contains 1.05% methionine)	314	267	−11
	III	Amino acid mixture + 1.05% methionine	261	287	10
	II A	4% casein (contains 0.14% methionine)	94	137	30

gradually extended to link up with each other so that there developed, throughout the whole liver, a fibrous network which isolated the individual lobules.

Chemical analyses, performed on the livers obtained from the animals fed the 4% casein diet and killed as a control measure (Group II H, Table 3) revealed sharp increases in liver lipids and collagen. The presence of advanced cirrhosis in the livers of these rats was confirmed by the microscopic studies.

3. *Afterperiod.*—Body Weight Gain: The results presented in Table 4 indicate that the rats fed the 30% casein diet (Group II C) showed a 92% gain in body weight by the end of the 16 weeks of therapy, while those fed the basal amino acid mixture supplemented with 1.05%, 1.83%, or 0.14% DL-methionine with or without added choline chloride (i. e., Groups II B, II E, II F, II D) showed only 70%, 56%, 45%, and 27% weight gain, respectively, at the end of this interval.

Feeding the basal amino acid mixture free of methionine and choline resulted in symptoms of severe anorexia in all animals receiving this ration (Group II G, Table 4). By the end of the 12th week of the afterperiod, 11 of the 15 animals given this experimental diet had died. The four remaining animals showed an average loss in body weight of 29%, and were therefore killed at this time (Week 12 of the afterperiod).

Liver Weights: It is evident from the results presented in Table 5 that the cirrhotic animals fed the 30% casein diet (Group II C) or the amino acid mixture with 1.83% DL-methionine (Group II E) had the heaviest livers of all the experimentally treated groups on the basis of wet weight, dry weight, grams of liver per 100 gm. of body weight, or fat-free liver weight. The liver weights of these two groups compare favorably with data obtained for control groups fed either 30% casein (Group I) or the amino acid mixture with 1.05%

TABLE 5.—*Effect of Dietary Protein and Amino Acid Mixtures on Liver Size After Sixteen Weeks of Feeding* *

	Group No.	Ration	No. Animals	Liver Weight		Gm. Wet Liver/Gm. Body Wt. †	Dry Fat-Free Liver Wt., Mean ‡
				Wet †	Dry †		
Experimentals	II D	Amino acid mixture + 0.14% methionine	10	5.39±0.21	1.78±0.07	2.76±0.21	1.14
	II B	Amino acid mixture + 1.05% methionine	11	5.33±0.13	1.74±0.06	2.36±0.21	1.37
	II C	30% casein (contains 1.05% methionine	13	7.80±0.26	2.34±0.07	3.00±0.07	1.93
	II E	Amino acid mixture + 1.83% methionine	14	7.65±0.14	2.30±0.07	3.76±0.09	1.86
	II F	Amino acid mixture + 0.14% methionine	10	5.45±0.17	1.63±0.02	2.65±0.06	1.32
Controls	II H §	4% casein (contains 0.14% methionine	18	6.32±0.34	2.81±0.09	4.68±0.62	1.01
	I	30% casein (contains 1.05% methionine)	5	6.89±0.33	2.29±0.08	2.40±0.31	1.85
	III	Amino acid mixture + 1.05% methionine	10	6.95±0.11	2.40±0.12	2.26±0.35	1.93
	II A	4% casein (contains 0.14% methionine)	2	4.28±0.43	1.94±0.00		0.82

* The experimental animals previously were fed standard 4% casein diet for 16 wk.
† Values represent mean ± standard error.
‡ Values represent mean.
§ Killed at end of foreperiod.

TABLE 6.—*Effect of Dietary Protein and Amino Acid Mixtures on Liver Lipids After Sixteen Weeks of Feeding* *

	Group No.	Ration	Liver Fat, % Dry Wt. †
Experimentals	II G	Amino acid mixture; no methionine	23.3±2.14
	II D	Amino acid mixture + 0.14% methionine	36.1±1.76
	II B	Amino acid mixture + 1.05% methionine	21.5±0.92
	II C	30% casein (contains 1.05% methionine)	17.7±0.51
	II E	Amino acid mixture + 1.83% methionine	19.1±0.89
	II F	Amino acid mixture + 0.14% methionine + 0.50% choline	18.8±0.75
Control	II H ‡	4% casein (contains 0.14% methionine)	64.3±1.98
	I	30% casein (contains 1.05% methionine)	19.2±0.87
	III	Amino acid mixture + 1.05% methionine	19.7±0.19
	II A	4% casein (contains 0.14% methionine)	58.0±4.41

* The experimental animals previously were fed standard 4% casein diet for 16 wk.
† Values represent mean ± standard error.
‡ Killed at end of period.

DL-methionine (Group III) for the entire 32 weeks of the experiment.

Liver Lipids: In rats fed 30% casein and in those rats fed an amino acid mixture with 1.83% DL-methionine during the 16 weeks of therapy, the concentration of lipid in the livers was comparable to that of animals fed the two nutritious control diets for the entire 32 weeks (Table 6). In general the concentration of lipid increased as the amount of methionine in the diet decreased. The addition of choline to an amino acid mixture low in methionine exerted a marked lipotropic effect (Group II F). The livers of animals fed 4% casein throughout the experiment had the highest concentration of fat (Table 5).

The mean liver lipid values for rats killed after 12 weeks of feeding the basal amino acid mixture free of methionine (Group II G) was 23.5% dry weight. This value is lower than that obtained when 0.14% DL-methionine was incorporated into the basal amino acid mixture. The lower value probably is related to the impaired growth of Group II G.

Liver Collagen: Average liver collagen values of 6.84 and 7.55 mg. per gram of dry fat-free liver were obtained for the Control Group I (30% casein) and Control Group III (amino acid mixture containing 1.05% DL-methionine), respectively (Table 7). The value of 6.84 was taken to represent the normal level of liver collagen. In addition to being expressed as milligrams of collagen per gram of dry fat-free liver, collagen is expressed as total hepatic collagen because of the possible "dilution" error

TABLE 7.—*Effect of Dietary Protein and Amino Acid Mixtures on Liver Collagen* *

	Group No.	Ration	(1) Dry Fat-Free Liver Wt. †	(2) Liver Collagen, Mg./Gm. Dry Fat-Free Liver	(3) Total Hepatic Collagen at Autopsy ‡ §	(4) Total Hepatic Collagen if Fibrosis Had Not Occurred (6.84 × Liver Wt.) †	(5) Amount of Collagen Due to Fibrosis (Col. 3—Col. 4)
Experimentals	II D	Amino acid mixture + 0.14% methionine	1.14	18.98±2.34	21.68±2.65	7.79	13.87±2.59
	II B	Amino acid mixture + 1.05% methionine	1.37	12.84±1.0	17.59±1.47	9.37	8.22±1.18
	II C	30% casein (contains 1.05% methionine)	1.93	8.10±0.81	15.63±0.22	13.20	2.43±1.27
	II E	Amino acid mixture + 1.83% methionine	1.86	10.75±0.74	19.99±1.28	12.72	7.27±1.42
	II F	Amino acid mixture + 0.14% methionine + 0.50% choline	1.32	13.40±1.67	17.68±2.19	9.03	8.65±1.73
Controls	II H	4% casein (contains 0.14% methionine)	1.01	21.00±3.88	21.21±4.11	6.91	14.30±4.17 (after 16 wk.)
	I	30% casein (contains 1.05% methionine)	1.85	6.84±0.63	12.65±1.31	12.65	----
	III	Amino acid mixture + 1.05% methionine	1.93	7.55±0.55	14.57±0.24	13.19	----
	II A	4% casein (contains 0.14% methionine)	0.82	30.52±2.01	25.02±2.01	5.61	19.41±1.23 (after 32 wk.)

* The experimental groups previously were fed standard 4% casein diet for 16 wk.
† Values represent mean (dry fat-free liver tissue).
‡ Values represent mean (dry fat-free liver tissue) ± standard error; total liver collagen on basis of liver weight at autopsy.
§ Killed at end of 16 wk. foreperiod.

which may have occurred with parenchymal regeneration. The amount of collagen due to fibrosis (Col. 5, Table 7) was estimated by subtracting the "calculated" normal collagen of the total liver (6.84 times liver weight) from the total hepatic collagen found at autopsy (Cols. 3 and 4, Table 7). The results show that considerable amounts of collagen were present in the livers of rats fed the 4% casein diet and then killed at 16 weeks (Group II H). Prolongation of the 4% diet through the treatment period resulted in an even greater increase in collagen content (Group II A). Increasing the amount of DL-methionine in the amino acid mixture to 1.05% caused a significant drop in the collagen content. Increasing the DL-methionine further to 1.83% caused another decrease but of only slight degree. The addition of 0.50% choline chloride to the suboptimal 0.14% DL-methionine supplement caused an effect comparable to the 1.05% DL-methionine level. The reason for this is not apparent. The liver collagen content of animals given the 30% casein regimen most closely approximated the values of the control groups maintained on nutritious diets throughout the experiment. The 30% casein diet was significantly superior to any of the other dietary regimens in promoting repair and regeneration of the cirrhotic liver (Table 7).

Histologic Changes: The histologic appearance of the livers of animals given the various therapeutic regimens was appreciably changed when compared with the biopsy specimens taken 16 weeks previously. The amount of stainable lipid in the liver was greatly decreased or had disappeared at necropsy in the animals of each group except those which received the amino acid mixture with 0.4% DL-methionine (Group II D). The parenchymal cells at the time of biopsy showed considerable variation in size and shape and staining quality with the result that the liver cords appeared irregular and disarranged. After 16 weeks on the therapeutic regimens the liver cells tended to become uniform in appearance and to assume the regular normal pattern within the lobule. The fibrous tissue proliferation noted at the time of biopsy was altered in the sections at autopsy in that it had contracted and had become more compact and less edematous. This change makes an estimation of the amount of collagenous tissue exceedingly difficult. Nevertheless, it was felt that there was actual reduction in the amount of connective tissue seen at autopsy in the livers of certain animals which received 30% casein and the amino acid mixtures containing 1.05% or 1.83% DL-methionine. No differences were observed in the appearance of the livers in the latter three groups.

Comment

In the present experiments moderate and even advanced cirrhosis seemed to respond to dietary therapy with the disappearance of fat, the restoration of liver architecture, and the return of normal-appearing parenchyma. As observed previously by Gyorgy and Goldblatt,[6] it is difficult to decide "whether the regression of fibrosis as determined by histologic appearance in sections obtained at necropsy, was real or due more to a stretching and 'thinning out' of the originally wide fibrotic bands in the cirrhotic liver." On the basis of histologic findings in this series, the apparent decrease of connective tissue has been impressive. If values for collagen may be taken as the chemical equivalent of connective tissue, the difference in chemically determined collagen is further evidence for regression of fibrosis.

Reports by other workers [7,19,20-23] as well as the present studies suggest that substances besides choline and methionine may provide lipotropic activity. Recent reports have implicated lysine, tryptophane, and threonine; however, the presence of dietary choline is said to be essential for this effect of threonine. Since the diet of the experimental group in question (Group II D) was free of choline, it may be that amino acids other than threonine and methionine were responsible for the decrease in liver lipid.[26]

The present studies suggest that adequate dietary methionine may be an important limiting factor for repair of cirrhosis and regeneration of liver cells. Animals fed the basal amino acid mixture supplemented with only 0.14% DL-methionine had the smallest livers and showed no decrease in hepatic connective tissue nor other reparative changes (Group II D). Whether limiting the dietary level of an essential amino acid other than methionine would result in the same poor response appears worthy of investigation.

It appears that lipotropism and liver regeneration are distinct phenomena; first, liver lipid metabolism,[24] depending on the availability in the diet of such lipotropic factors as choline and methionine, and second, liver repair and regeneration, depending on the presence in the diet of a protein source consisting of a properly balanced mixture of amino acids in which methionine is present in an adequate amount. It appears likely that the lipotropic role of methionine takes priority over the demands for liver repair and regeneration, since the lipotropic effect of methionine and choline is observed whether liver regeneration occurs or not (Group II D, Table 7).

Evidence of this relationship also is found in the experimental animals (Group II F) fed the basal amino acid mixture containing a 0.14% supplement of DL-methionine and a 0.50% supplement of choline chloride. In this group, liver lipids decreased to normal levels presumably as a result of the lipotropic effect of the added choline (Table 6). The significant improvement in regeneration i. e., in terms of dry fat-free liver weight, noted (Table 5) in the livers of these animals as compared to the livers of the animals (Group II D) fed a similar dietary regimen but free of choline may represent the "sparing" of essential methionine as a lipotropic agent by choline, the net effect being a greater availability of the dietary methionine. Further evidence for this interpretation is the fact that feeding the basal amino acid mixture supplemented with 1.83% DL-methionine (Group II E) resulted not only in an arrest and possible diminution of the cirrhotic process and the return of liver lipids to normal but also in a stimulation of liver regeneration as measured by liver weight. This group had the heaviest livers of all the experimentally treated groups with the exception of that fed the 30% casein diet.

A recent report by Rutman and co-workers [25] indicates that "the effect of changing the protein content of the diet on the ability of slices from the livers of rats to incorporate labeled methionine (synthesize protein) is very significant, the highest rate of incorporation being that on a 45 percent casein diet and the lowest on a protein free diet. The rate on the high protein diet is twice that on the protein free diet." The present findings appear to support these observations and suggest that supplementation of the diet with methionine is of value in nutritional cirrhosis when this essential amino acid is administered in the presence of optimal amounts of the other amino acids.

The finding that the 30% casein diet resulted in a growth response which was significantly greater than what was observed after feeding the various amino acid mixtures is in agreement with observations of other investigators.[27,28] Since dietary proteins are digested relatively slowly, the absorption of the amino acids takes place gradually according to the rate of enzymatic liberation. Feeding amino acid mixtures may result in differences in the rates of intestinal absorption of the individual amino acids.[29,30] Even where amino acid mixtures of optimal composition are fed, the various amino acids may not be present in the tissues in such relative optimal concentration simultaneously as required for protein synthesis.[31] An intact protein appears to be nutritionally superior to amino acid mixtures.

Summary and Conclusions

Extensive fatty and fibrotic changes in the livers of rats produced by means of a low-

protein diet (4% casein) were arrested and corrected by feeding diets containing 30% casein or amino acid mixtures simulating 30% casein.

An amino acid mixture containing 0.14% DL-methionine (equivalent to 4% casein) exerted lipotropic action but little or no effect upon repair or regeneration of the cirrhotic liver. The addition of choline to the above mixture of amino acids seemed to provide a methionine-sparing action.

Feeding amino acid mixtures containing 1.05% or 1.83% DL-methionine (equivalent, respectively, to diets with 30% and 50% casein) resulted in decreased liver fat, regression of fibrosis, and regeneration of liver tissue.

An intact protein (casein) is superior to amino acid mixtures in promoting repair and regeneration of the livers of rats with dietary cirrhosis.

The present studies suggest that factors governing fatty changes in experimental dietary cirrhosis and those affecting liver regeneration may not be the same and may not necessarily be related.

Optimal repair and regeneration of the cirrhotic liver depends upon the presence in the diet of a balanced source of protein, in which methionine is a limiting factor but not the only one.

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A Clinical Note on Marchiafava-Micheli Disease

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The purpose of this report is threefold. Firstly, these data reemphasize the chronicity of the disorder, paroxysmal nocturnal hemoglobinuria (PNH). Secondly, this case illustrates a clinical recovery with the persistence of the underlying erythrocyte abnormality. Thirdly, and most important, it demonstrates that routine hematological studies may fail to suggest the presence of this entity. Therefore, it seems logical to suspect that this condition is commoner than generaliy considered. At the time this case was first studied and published,[1] there had been one other reported in the American literature.[2] As the fundamental and meticulous work on this disorder by Ham [3] and Ham and Dingle [4] in the Thorndike Memorial Laboratory marked an early phase of many years of careful and original observations on the mechanisms of red blood cell destruction by Dr. W. B. Castle * and those who have had the privilege of associating with him,[5-11] it seems appropriate to record the following data.

Case History

The patient is a 50-year-old white married woman, the mother of two children, aged 18 and 16.

Family History.—The patient's mother and three aunts had carcinoma of the breast. Her father had carcinoma of the intestine.

Past History.—Noncontributory.

Present Illness.—At the age of 18 years this patient noted the passage of dark urine. When she was examined at that time,[1] pallor of the skin and mucous membrane was noted. The abdominal examination was negative. The urine was dark amber and contained a trace of protein. Ten months after the onset, laboratory studies showed a red blood cell count of 3,400,000 per cubic millimeter and a hemoglobin value of 49%. The white blood cell count was 4950 per cubic millimeter with a normal differential. The patient noted that her urine was very dark when voided at night and early morning hours. Frequently, but not invariably, these episodes were more marked several days prior to her menstural periods. Two months later auto- and isohemolysins were thought to be transiently demonstrated during exacerbations of hemolysis. Anisocytosis and poikilocytosis were observed, and the reticulocyte count was 10%. The Donath-Landsteiner test was negative. Three years after onset the patient developed phlebitis of the left saphenous vein. At that time the hemoglobin ranged from 30% to 60%. There were occasional episodes of right lateral abdominal pain. Five years after onset the patient for two months had an acute psychosis from which she recovered. Three and one-half years later a severe exacerbation was associated with a temperature elevation up to 102 F and icterus. The red blood cell count dropped to 1,880,000 per cubic millimeter and hemoglobin to 5 gm. per 100 cc. The hematocrit was 18.4%. The white blood cell count was 2950, and the platelet count was 109,000 per cubic millimeter. Reticulocyte value was only 1%. The osmotic fragility showed that hemolysis began at 0.48% and was complete at 0.35%. Later a urinary tract infection caused recurrent febrile episodes. One was associated with a marked fall in hemoglobin to 25% and hemoglobinuria. Urinary sediment showed hemosiderin, and the stool exhibited an excess of urobilinogen. Twelve and one-half years after onset the patient had a forceps delivery of a normal child. The red blood cell count at that time was 2,580,000 per cubic millimeter, with a hemoglobin of 8.7 gm. per 100 cc. The white blood cell count was 6050 per cubic millimeter, and the icterus index was 7.5. One urine specimen was described as amber and showed a 2+ proteinuria. Two years later, at the time of delivery of the patient's second infant, the hemoglobin was 12.5 gm. per 100 cc. and the urine was benzidine-negative. Nine months later a spontaneous complete abortion occurred. This was unassociated with any clinically detected exacerbation of hemolysis. Seventeen years after the onset of the disease and during the 29th week of her fourth pregnancy a radical mastectomy was performed for medullary carcinoma. A therapeutic abortion followed. The hemoglobin was 8.2 gm.

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* " . . . before honor is humility," Proverbs, XV, 33b.

per 100 cc., and the white blood cell count was 8450 per cubic millimeter. Seven years later, at the time of an open reduction of a comminuted fracture of her left humerus, the patient's hemoglobin was 12.8 gm. per 100 cc. and the white blood cell count was 6050 per cubic millimeter.

At the time of the patient's first office visit, in May, 1957, she stated that she had noticed no symptoms of her disease in the intervening eight years. The urine had not been dark or red at any time. She had observed no weakness or icterus. No episodes of abdominal pain seemed ascribable to the blood dyscrasia. There had been no recurrence of phlebitis. Her emotional status had been normal.

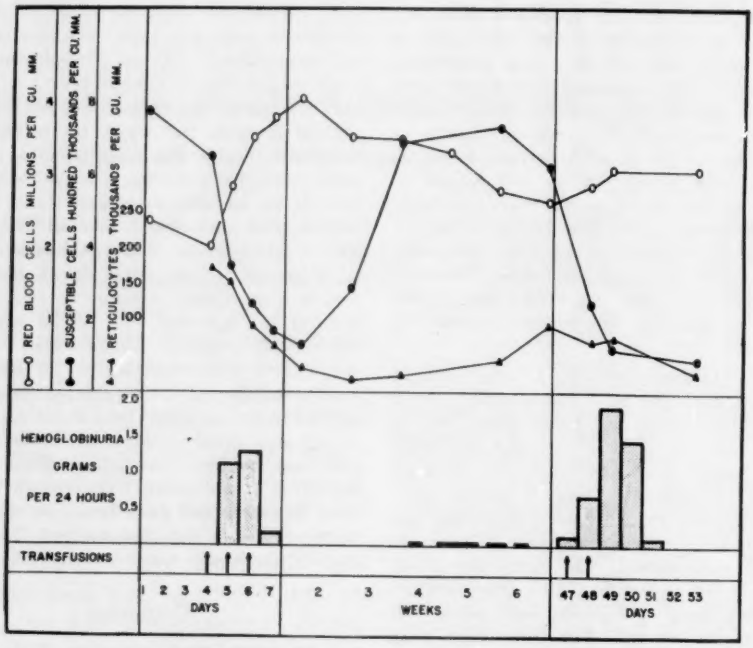
Physical Examination (May) 1957.—Height, 63¼ in. Weight, 174 lb. Blood pressure, 140/88 mm. of Hg. Pulse, 70. Temperature, 98.2 F. The skin was not remarkable except for healed surgical scars. The eyes were not unusually prominent. Conjunctivae were of normal color. The pupils were round, regular, and equal and reacted to light and accommodation. The media and fundi were normal. The extraocular movements were well performed. The external auditory canals were clear. The drums were intact. Air conduction was normal. The nares were clear. The nasal septum was intact. There was poor dental hygiene and an upper bridge. The gums appeared rather retracted. Findings on examination of the pharynx were benign. The tongue protruded in the midline without tremor. There was no papillary atrophy. The neck was supple. Cervical vessels were not unusually distended. The thyroid was palpable. The cervical lymph nodes were not enlarged. The trachea was in the midline. The left breast was absent. No nodules were seen in the area of skin grafting. There were no palpable axillary nodes. The right breast was normal. There was no unusual precordial activity. The precordial dullness was not increased. The heart sounds were of good quality. There were no murmurs. The rate and rhythm were normal. Chest expansion was equal bilaterally. The lungs were clear to percussion and auscultation. The diaphragms descended well. There was no unusual tenderness over the spine. Abdominal examination revealed no palpable masses. The liver and spleen could not be felt. There were no herniae. The neurological examination was negative. There was slight limitation of flexion in the left elbow, dating from her previous trauma. There was no edema of the left arm.

Laboratory Studies (May, 1957).—Hemoglobin was 16 gm. per 100 cc. The red blood cell count was 5,140,000, and the white blood cell count, 8300 per cubic millimeter. The hematocrit was 47. Platelets were 189,000 per cubic millimeter. A differential count showed segmented neutrophils, 54%; lymphocytes, 41%, and monocytes, 5%. The reticulocyte count was 1.1%. The urine was clear and straw-colored, with an alkaline reaction and a specific gravity of 1.013. There was 1+ proteinuria. There was no reducing substance. On microscopic study there were six to eight white blood cells and a few epithelial cells per high-power field. The urine was negative for urobilinogen. Serum bilirubin levels were direct, 0.31 mg., indirect, 0.06 mg., with a total of 0.37 mg. per 100 cc. On addition of a freshly made solution of 1% potassium ferrocyanide and 1% hydrochloric acid to the urinary sediment microscopic study revealed the epithelial cells to be loaded with blue-staining granules. An aliquot of the serum was layered with ether, and a 0.1 vol. of ammonium sulfide was added. This sample of treated serum had an absorption band at 558 mμ., indicating the presence of an ammonium hemochromogen, presumably derived from methemalbumin.[12] Sterile incubation of the patient's defibrinated blood for three hours revealed gross hemolysis. No cold or warm agglutinins were demonstrated. The osmotic fragility curve was within normal range.

Comment

The clinical manifestations of this disease have been carefully and critically described by Crosby [13] and Dacie.[12] A few pertinent points warrant attention. In the present case, 32 years after onset, the patient had no symptoms referable to her disease. No dark urine had been noted for several years, and on careful questioning no episodes suggestive of paroxysms of increased hemolysis could be recalled in the preceding eight years. No routine hematological laboratory study suggested the presence of the disease. However, methemalbumin was demonstrable in the serum; sterile incubation of defibrinated blood showed marked hemolysis, and hemosiderin-laden cells in the urinary sediment were numerous. Dacie [12] has described a case (Case 34) with apparent recovery of the patient from the disease. In that instance all laboratory studies yielded normal results except for leukopenia.

The mechanism of hemolysis in this disease is not clearly understood. The observations by Ham [3] and Dacie [12] have emphasized the probable importance in vivo of the lowering of pH in certain parts of the vascular bed. Such enhancement of lysis



Changes in hemoglobinuria and total red blood cell, "susceptible cell," and reticulocyte counts are shown in relation to repeated transfusions of compatible blood.[14]

by lowering pH is not unique, for it is evident in other hemolytic systems. The percentage of cells lysed by normal serum at a lowered pH (i. e., "susceptible cells") † varies from patient to patient. One (PFW) [14] of us has seen a patient with 28% of the red blood cells susceptible to lysis on repeated incubation in acidified normal human serum. The patient's red blood cell count was 3,300,000 per cubic millimeter. Another patient had 58% "susceptible cells," with a red blood cell count of 1,250,000 per cubic millimeter.

A possible relationship between the number of "susceptible cells" and hemoglobinuria was suggested by observations on a fourth patient [14] (Graph). Before the first series of transfusions the patient's "susceptible cells" ranged from 780,000 to 620,000 per cubic millimeter. The reticulocyte level was 152,000 per cubic millimeter, and the initial red blood cell count was 2,000,000 per cubic millimeter.

In a period of 48 hours the patient was given 1500 cc. of whole citrated blood. Twelve hours after the first transfusion the patient's "susceptible cell" count had fallen to 340,000 per cubic millimeter. The reticulocyte count remained at 150,000 per cubic millimeter, and the red blood cell

† "Susceptible cells" have been determined as follows.[14] Measured amounts of saline suspensions of red blood cells were centrifuged and resuspended in fresh human serum previously acidified by the addition of 5% (by volume) of N/3 HCl. These suspensions were incubated at 37 C for 45 minutes, then centrifuged and the acidified human serum changed. The suspensions were again incubated and the process repeated until no further hemolysis occurred. In control observations identical procedures were carried out using heat-inactivated acidified human serum. Heat-inactivated serum was prepared by heating 2.5 cc. aliquots at 57 C for 15 minutes. The cell suspensions were brought to their former equal volumes in saline. Red blood cell counts were then made. The difference between the number of cells in the control and test set-ups were expressed as percentages of the control. This percentage difference represented the cells destroyed by the procedure, i. e., "susceptible cells."

count had risen to 2,760,000 per cubic millimeter. In the 24 hours following the transfusion the patient excreted 1130 mg. of hemoglobin in the urine. As shown by the graph, the "susceptible cells" continued to decrease in number as the total red blood cells rose following two more transfusions. The reticulocyte count after the first post-transfusion 24-hour period began to drop. Hemoglobinuria remained high for 24 more hours, then decreased to small amounts during the succeeding 24 hours and subsequently disappeared for several weeks. For over three weeks the red blood cell count was well maintained and no reticulocytosis occurred. Concomitantly, after a two-week period, the number of "susceptible cells" began to increase. After their number had risen to the range of 600,000 per cubic millimeter, hemoglobinuria reappeared in traces. The total erythrocyte count gradually fell. When a level of 2,700,000 per cubic millimeter was reached, the reticulocyte count began to rise. Two transfusions of 500 cc. each were again followed by marked hemoglobinuria. No chills and only slight rise in temperature were associated with them. Again the number of "susceptible cells" fell markedly. These observations and the studies by Hinz et al.[15] indicate that the character of the cell population is one of the factors controlling the clinical manifestations.

The response of patients with PNH to transfusion has received much study, and it has been suggested that activation of the clotting mechanisms may inactivate a normal plasma inhibitor for this system (the so-called "stable" one because of its heat resistance), thus allowing lysis to occur.[13] Although AC-globulin[16] and thrombin[17] were suggested as participitating in the hemolysis of PNH red blood cells, subsequent observations by Harris et al.[18] demonstrated AC-globulin was not essential for such lysis. After dialyzing serum against saline the hemolytic activity of the former was lost but AC-globulin remained. Conversely AC-globulin could be permanently reduced in dialyzed serum previously treated with barium sulfate, while PNH activity could be restored by adding magnesium. Incubation of stroma of PNH cells with serum at a pH 6.5 liberated nitrogen. This activity was magnesium-dependent. Their results were interpreted as indicating the serum factor lysing PNH cells was a metal-requiring proteinase. Later Hinz et al.[19] pointed out several interesting correlations between the effect of zymosan and PNH red blood cell stroma. Stroma from such cells, like zymosan, inactivates a component of complement (C'_3) at 37 C. Pillemer and his associates[20] had previously shown that such inactivation required complement and magnesium and was due to a euglobulin-zymosan complex. The protein complexing with zymosan is properdin. It is distinct from complement and components of the clotting system. Serum from which this protein has been removed is not active against PNH cells, nor is properdin alone lytic in its effect on such cells. However, the reconstitution of such serum with properdin will restore its hemolyzing capacity for PNH cells. It is of interest that there may be a correlation between the enhancing effect of thrombin on the lytic activity of serum in this system and the amount of properdin contaminating thrombin preparations. Presumably the activation ascribed to thrombin in this hemolytic system may be due, partially at least, to the protein, properdin.[20] The properdin levels during crisis in three patients with PNH have been found to be low.[15]

Two factors have been accepted generally as the controlling elements of hemoglobinuria: (1) the amount of hemoglobin released intravascularly per unit of time exceeding that catabolized elsewhere and (2) the "renal threshold" for hemoglobin. The variation from person to person in the degree of hemoglobinuria with comparable levels of hemoglobinemia has been ascribed to differences in "renal threshold."[21] Glomerular permeability, glomerular filtration rate, and the tubular reabsorbing capacity have been considered variables

determining this threshold.[22,23] It has been repeatedly demonstrated in dog [24,25] and man [21,26] that exceeding the "renal threshold" has an immediate lowering effect upon it. Experimentally it may drop as much as 60%. However, ascribing such variability to alterations in renal function alone may not be warranted. Some evidence suggests "free" hemoglobin may be bound by a group of plasma mucoproteins to which the term haptoglobin has been given.[27] In the presence of excessive amounts of hemoglobin the haptoglobin is said to become saturated and the hemoglobin-haptoglobin complex apparently cleared from the plasma without being excreted in the urine. Thus, the haptoglobin level may transiently decrease according to Laurell and Nyman and in states of chronic hemoglobinemia the binding capacity of plasma for hemoglobin may diminish.[27] If these observations are substantiated, the amount of hemoglobin actually passing the glomeruli may vary in a manner that the burden on the tubules is not reflected simply in the degree of hemoglobinemia. The amount filtered may depend also on the amount of plasma haptoglobin present.

Conclusion

A case of Marchiafava-Micheli disease is presented. After a course of 32 years the routine clinical studies do not reveal this condition, although special observations show the abnormality of the red blood cells persists. The presence of the disorder in the absence of any unusual results on routine hematological study suggests that the disease may be commoner than generally considered. A few comments are made pertinent to the mechanism and clinical manifestations of this blood dyscrasia.

9 E. Chase St. (2).

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Biologic Turnover Rate of Cyanocobalamin (Vitamin B_{12}) in Human Liver

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The availability of γ-emitting radioactive cyanocobalamin (vitamin B_{12}) Co^{60} with a relatively slow rate of radioactive decay has made possible an estimate of the biologic turnover-rate of cyanocobalamin in the liver of man. There are several previously reported observations which suggest that this rate might be much slower for cyanocobalamin than for some of the other water-soluble vitamins, such as thiamine, nicotinic acid (niacin), and ascorbic acid. The interval between total gastrectomy and the development of megaloblastic anemia is usually several years.[1,2,3] Studies in pernicious anemia have shown that the interval between cessation of therapy and appearance of relapse will be greater than 6 months in 50 to 70% of the cases and in excess of 12 months in 25% of the cases.[4,5] Pitney and Beard [6] measured the serum cyanocobalamin level of a patient for 18 months after total gastrectomy, and they found that the level fell to one-half of the original value in about 9 months.

The liver is a large organ with a relatively high concentration of cyanocobalamin, and after the ingestion of radioactive cyanocobalamin there is a readily measurable quantity of radioactivity over the area of the liver.[7] According to Glass, Gellin, and Stephenson,[7] the radioactivity in the liver 90 days after the parenteral injection of cyanocobalamin Co^{60} was 86% to 94% of the peak radioactivity, which was observed 5 days after the injection. The rate of disappearance of this radioactivity in excess of the rate of physical decay of Co^{60} is a measure of the biologic half-life of hepatic cyanocobalamin. Implicit in the preceding statement is the assumption that the Co^{60} detected over the liver is physiologically active as cyanocobalamin and not an inactive degradation product containing cobalt.

This is a report of observations on the rate of biologic disappearance of radioactivity deposited in the liver after the ingestion of cyanocobalamin Co^{60}. Data are also presented indicating that such radioactivity has the solubility and chromatographic characteristics of cyanocobalamin when extracted from homogenized liver with various solvents.

Methods

Observations were made on six male patients with pernicious anemia and one healthy man. The patients were receiving intramuscular injections of 30μg. to 100μg. of cyanocobalamin every three or four weeks as maintenance therapy. All subjects studied had received cyanocobalamin Co^{60} orally several months prior to the beginning of these observations. The patients with pernicious anemia were given intrinsic factor to promote absorption of cyanocobalamin Co^{60}. Radioactivity was measured with a conventional scintillation detector and scaler. Correction for radioactive decay of the Co^{60} in the liver was made by counting the reference sample of Co^{60} on each day that a subject was observed for hepatic radioactivity. The ratio $\frac{\text{liver counts per minute}}{\text{reference sample counts per minute}}$ at the time of the

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A report of some of these data was presented before the Sixth International Congress of the International Society of Hematology, Boston, Aug. 27-Sept. 1, 1956.

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initial recording of liver radioactivity was designated as 100% hepatic radioactivity. Subsequent values for per cent hepatic radioactivity were calculated by the following formula:

$$\frac{\frac{\text{Liver counts per min. on day } T}{\text{Reference sample counts per min. on day } T}}{\frac{\text{Liver counts per min. on day } 0}{\text{Reference sample counts per min. on day } 0}} \times 100$$

The values computed by means of this formula were plotted on semilogarithmic paper, as in Figure 1.

To determine if the radioactivity observed over the liver of the patients represented intact cyanocobalamin Co^{60}, the following studies were made: A 1500 gm. liver, from a patient who had received cyanocobalamin Co^{60} 11 months prior to death due to chronic lymphocytic leukemia, was homogenized in a high-speed mixer,* digested with papain,[8] extracted with a mixture of cresol and carbon tetrachloride, and then reextracted with water.[9] The aqueous extract was passed through Amberlite IRA 400 resin[10] and extracted again with cresol-carbon tetrachloride and then with water. Aliquots from the several steps in this extraction procedure were tested for radioactivity and cyanocobalamin content.

Assays of cyanocobalamin activity were made by use of Lactobacillus leichmannii as the test organism.[11] †

The final aqueous extract of liver was subjected to ascending chromotography on Whatman No. 1 paper with use of 2:4 lutidin and water (14:10) as a developing agent in an atmosphere of ammonia.

* Omnimixer, Ivan Sorval.

† These assays were furnished by P. Derse and L. Hein, of the Wisconsin Alumni Research Foundation.

Results

The biologic half-life of hepatic radioactivity after the oral ingestion of cyanocobalamin Co^{60} approximates 400 days. The

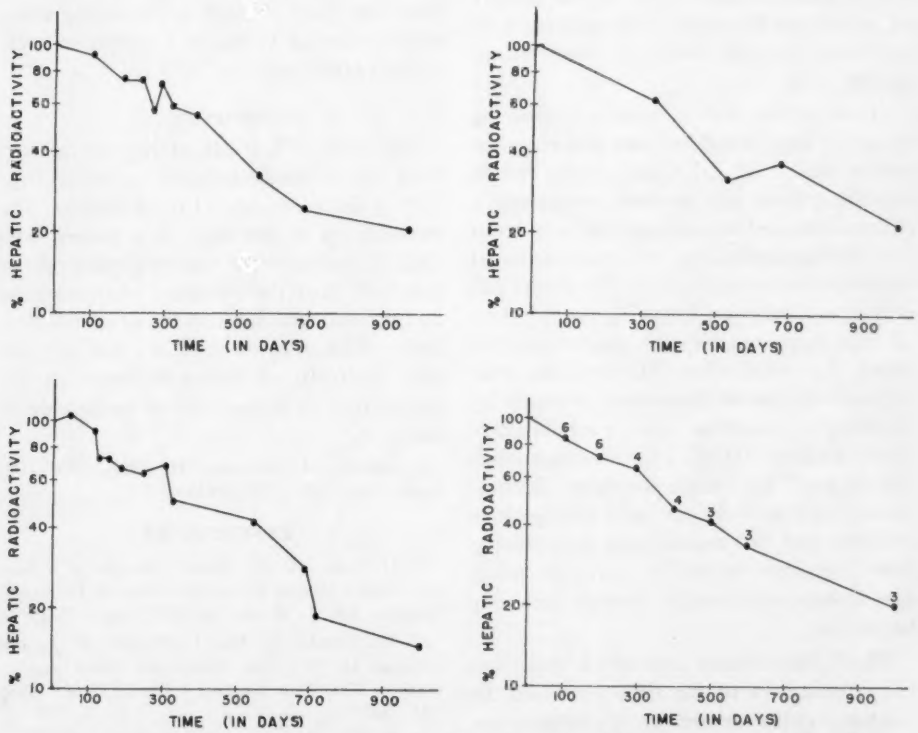

Fig. 1.—Biologic rate of disappearance of hepatic radioactivity after ingestion of radioactive cyanocobalamin. Radioactive decay has been eliminated from this figure. Upper, two patients who had pernicious anemia and were receiving maintenance injections of cyanocobalamin. Lower left, disappearance rate in a healthy man. Lower right, composite curve (five patients and one normal subject). The numbers above the respective points indicate the number of persons observed for that length of time.

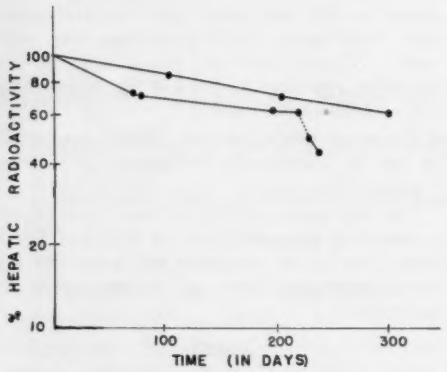

Fig. 2.—See legend for Figure 1. The dotted-line portion of the lower curve represents the rapid fall in hepatic radioactivity when the patient received cyanocobalamin in a dosage of 1 mg. intramuscularly twice a day for seven days (see text). The upper curve is the composite curve from the lower right portion of Figure 1.

rate of disappearance in the normal subject was similar to that seen in the patients with pernicious anemia receiving maintenance therapy (Fig. 1).

In one patient with pernicious anemia the decline in hepatic radioactivity was observed over a period of 217 days, during which time the patient was receiving maintenance cyanocobalamin therapy. For the next seven days he received 1 mg. of cyanocobalamin intramuscularly twice a day. The abrupt fall in liver radioactivity following the injection of this huge quantity of cyanocobalamin shows that even after 200 days the liver radioactivity can be diminished promptly by "flushing" injections of nonradioactive cyanocobalamin (Fig. 2). Doscherholmen and Hagen [12] have made analogous observations. Such data do not constitute positive evidence that the radioactivity disappearing from the liver is in the form of intact cyanocobalamin molecules, though such may be the fact.

The Table contains data which show that the radioactivity of the liver possessed the solubility characteristics of cyanocobalamin, because there is parallelism between radioactivity and microbiological cyanocobalamin activity during the several stages of extraction of the liver cyanocobalamin. Further

Extraction of Cyanocobalamin from Liver

Sample	Volume, Ml.	Cyanocobalamin,* μg./Ml.	Total Cyanocobalamin, μg.	CPM †/ μg. of Cyanocobalamin
Original liver ‡	1,500	1.08	1,620	3.7
Extract 1	4,000	0.37	1,480	3.9
Extract 2	6,000	0.20	1,200	4.2
Extract 3	600	1.77	1,062	4.4
Extract 4	130	6.39	831	2.6
Extract 5	154	2.42	377	4.2
Extract 6	10.5	27.3	286	4.1

Extract 1. Filtrate from papain-hydrolysed liver
Extract 2. Supernate after zinc defecation
Extract 3. Aqueous supernate after cresol-carbon tetrachloride extraction.
Extract 4. Aqueous extract after salting out with Na_2SO_4
Extract 5. Eluate after passing through anion exchange resin
Extract 6. Aqueous supernate after second cresol-carbon tetrachloride extraction

* L. leichmannii assay.
† CPM, counts per minute.
‡ The figure in Column 2 represents grams, and that in Column 3, micrograms per gram.

evidence that the radioactivity represents intact cyanocobalamin Co^{60} is the fact that the radioactivity and red color extracted from the liver showed a chromatographic mobility similar to that of a sample of crystalline cyanocobalamin.

Summary

The biologic half-life of hepatic radioactivity from cyanocobalamin (vitamin B_{12}) Co^{60} is approximately 11 to 14 months. The radioactivity in the liver of a patient who died 11 months after receiving cyanocobalamin Co^{60} had the solubility characteristics and chromatographic mobility of cyanocobalamin. This evidence indicates that the biologic half-life of cyanocobalamin in the human liver is of the order of magnitude of one year.

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Red Cell Lipids

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The great current interest in plasma fats and their relation to disorders of obvious clinical importance (e. g., arteriosclerosis) has tended to obscure in some degree certain other recent developments in knowledge of lipid metabolism. Among these, it is possible to record for the lipids of the red blood cell advances in our knowledge of their composition as well as a beginning of understanding of the dynamics of their metabolism. While it is too soon to appraise the importance of such researches for medicine, they appear to bear upon at least two old and interrelated problems of physiology.

The first of these concerns the architecture of the cell, especially its membranes. It has long been supposed that phospholipids have important structural functions, including those concerned with maintaining the characteristic cellular permeability. The second problem is that of metabolic activity, changes in which may be associated, for example, with hemolytic disease and aging of the cell. Since the study of the metabolism of red cell lipids has only just begun, it is impossible to say what its relation may be to these particular phenomena. However, the point can be made that, whereas the proteins of the mammalian erythrocyte (hemoglobin, stromatin) are largely inert, once having been formed,[1] lipids of several kinds are in a highly dynamic state—indeed, their biosynthesis at a lively rate is accomplished by the non-nucleated cell of the peripheral blood.[2] It is therefore entirely probable that their metabolic activities are no less vital than the more familiar ones of carbohydrates.

In work prior to 1940 it was shown that most of human red cell lipid is found in the stroma and that its composition is complex, including cholesterol, neutral fat, and glycolipid, as well as the phospholipids sphingomyelin, lecithin, and cephalin. The many difficulties associated with separation of the components, as well as uncertainties as to their homogeneity, left matters for a time in a rather unsatisfactory state, but the application of newer methods now promises considerable clarification. As a result of the investigations of Folch and others,[3] cephalin of brain has been shown to be a mixture of three phosphatides, and two of these, phosphatidyl serine and phosphatidyl ethanolamine, make up the bulk of the cephalin fraction of human and dog red cells.[4] Chromatography of red cell lipid extracts on silicated papers [5] has shown that the cephalin fraction also contains plasmalogens.[6]

The choline-containing phosphatides, sphingomyelin and lecithin, are present in human erythrocytes in about equal amounts.[7] However, the distribution of these substances in animal cells may show remarkable deviation from the human pattern. On the one hand, the cells of dog and guinea pig are characterized by relatively large amounts of lecithin with small amounts of sphingomyelin. On the other hand, the cells of the true ruminants (ox, sheep, goat) appear to contain much sphingomyelin but little or even no lecithin,[6] a substance hitherto presumed to be present in virtually all cells.

Species differences are also demonstrable for glycolipids, perhaps particularly as regards the kind of amino sugars found. Thus, in human stroma the predominant glycosphingoside contains galactose, glucose,

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and chondrosamine.[8] Glucosamine appears in the glycolipids of bovine cells,[8] and sialic (neuraminic, hemataminic) acid, in those of the horse.[9]

Analysis of the fatty acids of tissues has recently become both accurate and rapid with the development of gas-liquid chromatography by James and Martin [10] and its application to blood lipids by James, Lovelock, Webb, and Trotter.[11] A great variety of fatty acids may be found in the erythrocyte, but even more striking and important is the demonstration of the synthesis of lipids by the red cells of human peripheral blood.[2] It has been known for some years that erythrocyte cholesterol is in dynamic equilibrium with plasma cholesterol,[12] but the synthetic activity of the cells has been regarded as inconsequential. It now appears that upon incubation of whole blood in vitro with C^{14} acetate for a few hours, as much as 1% of the labeled substance may turn up as a constituent of the fatty acids of the phospholipids. These, as well as free cholesterol, move back and forth between cells and plasma. The neutral fat of the red cell is even more highly labeled but seems to travel in one direction only, i. e., out into the α-lipoprotein, and the red cell may be an important source of this plasma lipid.

This brief sketch of developments in the field of red cell lipids may serve to indicate the direction of current investigations. It is certain that within a short time the problems of red cell disease in human subjects will be explored, and there is good reason to think that abnormalities of lipid composition and metabolism will be uncovered.

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The Mechanism of Bone Marrow Repopulation

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Since the beginning of the awareness of the acute radiation syndrome which followed accidents in this country and the meager knowledge concerning the effects of the atomic bomb at Nagasaki and Hiroshima many attempts have been made to abort the syndrome by various means. Jacobson's [1] experiments in shielding certain of the hematopoietic organs and, later, Cole's [2] work in replacing the damaged cells with emulsified spleen pulp gave the earliest good results.

Lorenz [3] later began replacing the damaged bone marrow cells with similar undamaged cells.

Early in this work the mechanism of activity of the shielded spleen and transplanted cells brought about two hypotheses: (1) that humeral effects brought about the improvement and (2) that the transplanted cells were able to proliferate and mature and in the end bring about repopulation of the damaged organs of hematopoiesis.

In a review of the work on bone marrow transplantation, Congdon [4] has shown proof that bone marrow cells from other animals repopulate the marrows and blood-forming organs of animals that have received lethal doses of radiation. He discusses the possibility of humeral effects but seems of the opinion that the regrowth of the damaged organs is a matter of replacement.

All this work poses many questions. The first returns to the double question of whether there is humeral effect and whether this is divided between donor cells and the recipient animal or whether these cells are innately endowed with the ability to perform proliferation, maturation, and repopulation without help from the recipient host.

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Secondly, are there previous hints in other work that would lead to any other conclusions?

Thirdly, is there work which would cast a doubt on what is apparently a fact, that the donor cells react in an in vivo test-tube experiment not possible as yet in in vitro experiments?

Fourthly, what is known of the requirements for proliferation and maturation of hematopoietic cells that would explain the repopulation of the hematopoietic organs of lethally irradiated animals?

The answer to the first double question may be determined in two parts, first, by further experiment and, second, in an analysis of the answers to Questions 2, 3, and 4. The second and fourth questions may possibly be answered at the same time.

It would be well to turn to embryology and histology of the blood-forming organs for some clue as to what has occurred. The blood islands, for instance, are the earliest blood-forming sites. In these little or no differentiation occurs. It is only after lymphoid tissue begins to be formed that in the embryo granulocytes and non-nucleated red cells are found. Indeed, as lymph nodes and lymphopoiesis is found in the embryo myelopoiesis occurs along with it. Thus, it would seem that, although primitive proliferative myelopoiesis may occur without lymphopoiesis, proliferative myelopoiesis and or lymphopoiesis may not occur without their mutual reciprocity. It must be remembered, too, that the liver and spleen of the fetus in most animals carries on all hematopoiesis throughout the greater part of fetal life and that the bone marrow begins to function only late in fetal life.

The bearing that this has on the repopulation of the bone marrow of irradiated

animals may be somewhat subtle but nevertheless important. The marrow of rats and mice is made up to a large extent of undifferentiated cells, and much of the hematopoiesis of each animal is carried out in the spleen and, to a certain extent, in the liver. In some strains of mice granulopoiesis and erythropoiesis occur normally through adult life in lymph nodes. Neither lymphoid or myeloid maturation occurs without the other. A lethally irradiated animal has lost not only myeloid, or bone marrow, cells but lymphoid cells as well. Therefore, in saving the spleen by shielding or by injecting spleen cells or bone marrow cells from nonirradiated animals into irradiated animals the effect is that of giving both strains of cells.

Of importance, too, to the question of repopulation of the marrow of lethally irradiated animals is the field of tissue culture. The life-giving properties of living cells without the aid of humeral help must depend on the ability of a few billion cells to proliferate and mature without enzymatic or hormonal stimuli. If the lethally irradiated animal is considered as a test tube for tissue culture, the results in in vitro tissue cultures and in the animal experiment do not agree. In in vitro tissue cultures,[5] from the earliest continued explants to the later results, such as those described by Bierman,[6] little differentiation is shown in the continued explants.

Bierman described four phases through which explanted cells develop. In the first culture there may be some differentiation, but after several transplants, he states, the cells resemble no known blood or bone marrow cells if their origin has been from either. And yet, in animals that have been given lethal doses of radiation normal proliferation and maturation occur to the extent of repopulation of the bone marrow, even to the reoccurrence of myeloid cells in lymph nodes and spleen.

In earlier tissue culture work Benewolenskaja (cited by Bloom[5]) described granulopoiesis and erythropoiesis in explants of human embryo liver. To these explants had been added extracts of the same embryos. Such extracts must have contained both lymphoid and myeloid humeral substances. Maximow[5] reported transformation of lymphoid cells into myelocytes in explants of lymph nodes grown in plasma and bone marrow extract. He concluded that all granular cells originated from nongranular cells. This is accepted today by most authorities.

Other striking things happen in animals and humans who have received lethal doses of x-ray.[7] Not only are the blood-forming elements destroyed but much of the metabolism is disrupted. As well as this, there is considerable intestinal and gonadal cellular damage and frequently skin and hair changes.

There is some evidence that lethal irradiation may bring about adrenal[7] disturbances and that for several days after irradiation there may be at first increased amounts of 17-ketosteroids in the urines of such animals and later decreased amounts. The overproduction of adrenal hormones through the day of explant of the repopulating marrow cells might aid in proliferation and maturation of these cells.

Damage to the lymph nodes may also result in some humeral effect which would be advantageous to the donor cells. The reason why the marrow is the burial ground for a large number of lymphocytes, as cited by Drinker and Yoffey,[8] has never been adequately explained.

The work that began with a report by Wearn, Miller, and Heinle[9] and has been furthered largely by Miller and Turner[10] and their co-workers might well explain the humeral effect of repopulation. That is, if living cells are given a fertile medium in which to grow, with the necessary stimuli for proliferation and maturation, normal myelopoiesis and lymphopoiesis again occur in the irradiated animal.

Another explanation might be entertained. That is that Cantarow[11] in 1952 developed lymphosarcomas and leukemias in rats with

mitochondrial and chromatin particles. It is possible that, because the lungs act as barriers to cell emulsions, all bone marrow transfusions result in cell breakdown and the repopulation occurs from the mitochondrial and chromatin particles that are circulated.

Repopulation of the bone marrow of lethally irradiated animals following explants from normal animals occurs because either whole living cells are innately capable of proliferation and maturation or the irradiated animal supplies stimuli in the form of enzymes and hormones to bring about the double process of growth, i. e., proliferation and maturation. The former is unlikely because of the vast evidence of poor differentiation or maturation of the multiple transfer of explants of almost all types of cells. It is also still possible that cell particles such as mitochondrial and chromatin may bring about the repopulation. Further experiments are necessary to give the final answers.

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Hemoglobin Tolerance in Various Types of Anemia

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Introduction

In the majority of patients with chronic hemolytic anemia either of acquired or congenital type, reticulocytosis and persistent increases in bilirubin globin are the prominent characteristics. However, hemoglobinemia and hemoglobinuria are rarely found in these patients even during their hemolytic crisis, when a considerable amount of blood is presumably destroyed, as indicated by the rapid fall of the hemoglobin level and hematocrit in a relatively short period. On the contrary, however, in patients with acute hemolytic anemia the earliest and most outstanding findings are marked hemoglobinemia and hemoglobinuria, which are, as a rule, followed by an only temporary increase in bilirubin globulin in the the plasma and reticulocytes in the peripheral blood.

This discrepancy leads us to think that the cells of the reticuloendothelial system (perhaps the liver and the spleen of patients with chronic hemolytic anemia) may be capable of adapting to, or compensating for, the process of destruction of red blood cells by increasing the rate of hemoglobin metabolism or by conversion of hemoglobin to some other form which cannot be detected as free hemoglobin. In order to demonstrate the possibility of the existence of such a mechanism for dealing with the increased pigment formation, a study of hemoglobin tolerance was made in normal persons and in patients with chronic hemolytic and with other types of anemia. This was done by measuring quantitatively the hemoglobin and bilirubin in the plasma as well as the hemoglobin in the urine during and after the slow infusion of hemoglobin solution.

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From the Cancer Research and Cancer Control Unit, Tufts University School of Medicine, Department of Medicine and Surgery, and the Research Laboratory of the Holy Ghost Hospital.

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Method of Investigation

Adjustable Mechanical Pump.—Because of the uncontrollable variation of venous pressure, the regulation of the rate of infusion to the patient by the ordinary intravenous set-up is difficult. However, this problem is overcome by using a specially designed apparatus with a Fenwall intravenous set-up. This apparatus consists of a motor (½ horsepower), a rotator, and a gear box with an adjustable handle to regulate the speed of rotation (Fig. 1). The rotator (9 cm. in diameter) has six small wheels (2.6 cm. in diameter) attached to its

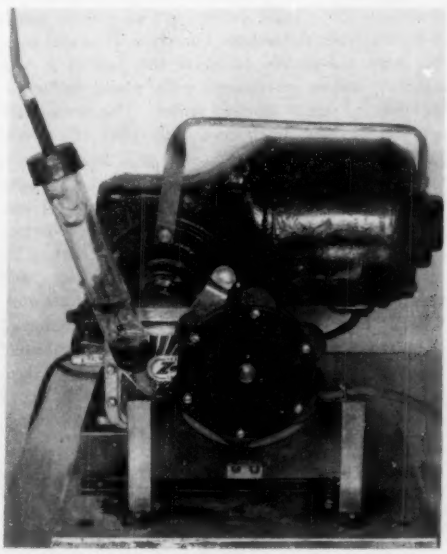

Fig. 1.—The adjustable mechanical pump.

circumference 1.8 cm. apart. Below the rotator is a narrow board which is supported by a spring on each side. The top of this board has a concave surface which is parallel to one-third of the circumference of the rotator. A channel (0.6 cm. in depth) is carved along this concave surface to fit the edge of the small wheels. Part of the rubber tube between the blood filter and the intravenous needle of the Fenwall set can thus be inserted into this channel under the small wheels by merely applying pressure on the concave surface of the board. As the rotator turns, the small wheel runs along the channel, milks the rubber tube, and forces the solution to flow toward the vein. Before the subsequent wheel milks the tube again, the part of the tube emptied by the first wheel has filled up with the solution from the bottle above by gravitational force. By this process, the number of drops per minute through the blood filter to the patient is regulated simply by adjusting the handle of the gear box which controls the speed of the rotator.

Previous to infusion, the amount of solution in each drop was estimated from the known concentration of the prepared solution and the number of drops in 10 cc. volume measured through the blood filter by the action of the pump. The amount of solution per minute required for infusion is thus calculated and then administered by the mechanical pump, which has been adjusted to the desired rate of speed. This apparatus permits the intravenous infusion of measured quantities of hemoglobin solution by maintaining a constant rate (from 0.5 cc. to 8 cc. per minute) regardless of variations in venous pressure.

Preparation and Administration of Hemoglobin Solution.—Fifty cubic centimeters of venous blood is drawn from the patient (or from a normal person with compatible blood if the patient is too anemic) and is introduced with sterile technique into 500 cc. sterile distilled water. This hemolyzed solution is then introduced into 1000 cc. sterile 1.25 gm. per 100 cc. NaCl, making a final tonicity of approximately 0.83 gm. per 100 cc. A sample of the hemoglobin solution is then poured off and spun for five minutes at 3000 rpm, and a hemoglobin determination is made on the supernatant by diluting 1 cc. with 20 cc. of distilled water and reading on an Evelyn photoelectric colorimeter. The hemoglobin in grams per 100 cc. is obtained from the chart, which is standardized and calibrated from a known hemoglobin solution of 1:500 dilution. The figure thus obtained is multiplied by 21 and divided by 500 to give grams of hemoglobin per 100 cc. of hemoglobin solution.

The flask containing the hemoglobin solution is fitted with a Fenwall infusion set, and the rubber tube is run through the pumping machine. Previous to infusion, the number of drops from the blood filter in 10 cc. volume is counted. The number of drops required to obtain 5.4 mg. of hemoglobin (the amount liberated per minute in a normal person) is then calculated.

During the first hour 0.325 gm. of hemoglobin was administered at a rate of 5.4 mg. per minute. This was approximately one-twenty-fourth the amount of hemoglobin contained in the red cells presumably destroyed by a normal person in 24 hours (7.8 gm. of hemoglobin). Thereafter, the amount of hemoglobin solution infused during each hour was increased by 0.325 gm. at the beginning of each subsequent hour, so that at the end of five hours a total of 5.29 gm. of hemoglobin had been administered. Observations were made of the plasma hemoglobin and bilirubin levels at the end of each hour for 5 hours and at the end of 2, 4, and 19 hours after the termination of infusion. The amount of plasma hemoglobin and indirect bilirubin measured at the end of each hour of infusion minus their initial values prior to infusion was the amount of the increase recorded.

Previous to the infusion and for 24 hours thereafter, urine specimens were collected separately. Urinalysis was done and the total amount of urine determined. The hemoglobin in the urine was measured, and the total amount of hemoglobin excreted was calculated. The patient was usually weighed either before or shortly after infusion.

Blood samples of 5 cc. each were collected from an antecubital vein of the other arm with a syringe and a sterile needle rinsed three times with 3.2% sodium citrate. The blood was transferred to a test tube containing 0.1 cc. of 3.2% sodium citrate solution and gently mixed. The sample was centrifuged immediately and the plasma separated with care. This plasma was again centrifuged and again separated to avoid any contamination with red cells. The venipuncture for drawing each blood sample should be performed carefully to minimize the possibility of traumatic hemolysis.

Quantitative estimation of the hemoglobin in the plasma and urine was measured colorimetrically by the method described by Ham.[1] The quantitative determination of serum bilirubin was performed by the method of Watson and Ducci,[2] with use of the Evelyn photoelectric colorimeter. Hemosiderin was detected microscopically as black-staining particles in a drop of sediment from centrifuged urine observed after the addition of a drop of ammonium sulfide solution.

The Ashby technique[3] was employed for the determination of the survival of transfused erythrocytes. The methods employed for estimating hemolytic activity in this investigation have been described elsewhere as follows: determination of osmotic fragility,[4] determination of mechanical fragility[5] of red blood cells, and test for Coombs titer.[6]

TABLE 1.—*Summary of Observations Made on Hemolytic and Nonhemolytic Patients*

Diagnosis	Body Weight, Kg.	Blood HB., Gm./100 Cc.	Plasma Hemoglobin Before Infusion, Mg./100 Cc.	Increase in Plasma Hemoglobin At End of Infusion, Mg./100 Cc.	Hemoglobinuria, Mg.	Increase in Indirect Bilirubin, Mg./100 Cc.	Survival of Transfused Cells in Pt., Days	Remarks
Hemolytic cases								
Acquired hemolytic anemia								
Case 1	83.6	9.1	1.45	10.4	0	0.21	27	Coombs titer (1:128)
Case 2								
Before splenectomy	65.9	5.1	4.68	23.0	0	0.68		Coombs titer (1:2048)
After splenectomy		9.7	2.47	17.6	0	0.47		Coombs titer (1:1024)
Case 3	91.0	4.5	2.05	33.9	0	0.42	30	
Case 4	83.4	4.7	0.83	23.5	0	0.47	49	Coombs titer (1:64)
Congenital hemolytic jaundice	59.3	8.6	1.52	19.0	0	0.10		Spherocytosis increased in O. F. & M. F.
Sickle-cell anemia								
Case 1	55.0	9.2	15.3	15.9	0	0.30		
Case 2	39.0	9.3	3.6	29.3	0	0.97		
Cooley's anemia	46.0	8.3	1.89	15.5	0	0.88		
Chronic myelogenous leukemia *	52.3	6.0	1.77	27.5	0	1.6	25	M. F. 9.5%
Myeloid metaplasia *								
Case 1	76.3	6.5	1.62	12.5	0	0.60	22	M. F. 10.1%
Case 2	72.0	9.6	0	25.2	0	0.52	30	M. F. 7.3%
Case 3	58.6	15.9	1.00	17.7	0	0.58	50	M. F. 4.5%
Cirrhosis of liver								
Case 1	60.0	6.8	1.70	32.6	0	0.65	55	
Case 2	92.3	8.6	3.0	22.0	0	1.59	60	
Case 3	55.0	11.9	2.07	32.9	0	.23	75	
Reticulum-cell sarcoma	54.5	6.5	1.55	26.2	0	0.40		M. F. 7.5%
Paroxysmal nocturnal hemoglobinuria								
Case 1	69.1	7.4	36.4	89.5	620.3	0.12		
Case 2	51.0	6.7	12.5	50.2	251.1	0.21		
Nonhemolytic cases								
Refractory anemia								
Case 1	77.0	7.2	2.07	55.8	0	0.78		
Case 2	59.1	8.6	0	35.9	27.8	0.11	95	
Hypochromic anemia								
Case 1	63.0	7.4	0	39.8	25.8	0.58		
Case 2	69.2	5.6	2.96	48.0	0	0.43		
Case 3 †	45.0	8.5	1.09	49.0	44.9	0.55	115	
Lymphoma	51.0	6.8	1.48	38.9	0	0.30	110	
Carcinoma of stomach	55.5	12.9	2.68	81.1	0	0.12		
Carcinoma of stomach (with metastases to liver)	59.5	10.2	4.14	40.5	0	0.70		
Carcinoma of esophagus	63.7	13.1	4.23	60.9	0	0.24	120	
Carcinoma of rectum (with metastases to liver)	61.7	7.8	2.43	49.4	0	0.62		
Carcinoma of larynx	66.0	13.2	2.33	100.9	0	0.59	110	
Carcinoma of breast	55.0	14.2	0	94.3	9.8	0.10	100	
Carcinoma of lung	54.6	11.1	1.60	57.0	0	0.12		
Carcinoma of prostate								
Case 1	52.5	11.8	2.70	47.4	0	0.30	90	
Case 2	61.3	9.7	0	38.2	0	0.47		
Case 3	52.0	10.7	0	70.1	0	0.32	120	
Multiple myeloma								
Case 1	77.3	13.9	0	66.4	0	0.16		
Case 2	51.0	5.8	5.42	45.4	0	0.33		

* With liver involvement.
† With Cooley's trait.

Results

Eighteen patients with hemolytic complications and eighteen nonhemolytic cases were studied. The diagnoses and a summary of the findings are listed in Table 1. Among the hemolytic group all but one had anemia and their hemoglobin levels varied from 4.5 to 11.9 gm. per 100 cc. The patient without anemia (hemoglobin 15.9 gm. per 100 cc.) was one of the cases of myeloid metaplasia who exhibited hepatomegaly, massive splenomegaly, and definitely shortened survival time of the transfused erythrocytes (50 days). All cases of the hemolytic group except those with unquestionable hemolytic diseases, such as paroxysmal nocturnal hemoglobinuria, sickle-cell anemia, or Cooley's anemia, had either a positive Coombs tests, increase in mechanical fragility, or shortened survival time of the transfused cells. Among the four cases of acquired hemolytic anemia, two showed positive Coombs titer of 1:128 and 1:64, with shortened survival time of the transfused red blood cells (27 and 49 days, respectively); one had positive Coombs titer of 1:2048, and the other one, with negative Coombs' test, had a definitely shortened survival time of transfused cells of 30 days.

In three cases of myeloid metaplasia, two had an increase in mechanical fragility of erythrocytes of 10.1% and 7.3%, with shortened survival time of transfused cells of 22 and 30 days, respectively; the third one had normal mechanical fragility of 4.5%, but the survival time of the transfused red blood cells was only 50 days. Three cases of cirrhosis of the liver showed shortened survival time of the transfused red cells of 55, 60, and 75 days. The case of chronic myelogenous leukemia and the case of reticulum-cell sarcoma exhibited an increase in mechanical fragility to 9.5% and 7.5%. The former case in addition had a short survival time of the transfused cells of 25 days.

In 18 cases of the nonhemolytic group, 5 had no anemia (hemoglobin 12.9 to 14.2 gm. per 100 cc.) at the time of study, one of whom had received a transfusion shortly before study and one after response to cyanocobalamin (vitamin B_{12}) therapy. The remainder had hemoglobin levels of from 5.6 to 11.8 gm. per 100 cc. All the usual studies of hemolytic activity made in this group were within normal limits. The survival study of the transfused cell was performed in eight of these cases. All were above 90 days.

At the end of the hemoglobin infusion, 16 of the 18 patients with hemolytic anemia and the 1 case of myeloid metaplasia without anemia had increases in the plasma hemoglobin level of between 10.4 and 32.9 mg. per 100 cc. The two patients with paroxysmal nocturnal hemoglobinuria who had high initial plasma hemoglobin levels of 36.4 and 12.5 mg. per 100 cc. had increases of 89.5 and 50.2 mg. per 100 cc., respectively.

In 13 of the patients with nonhemolytic anemia, the increases were between 35.9 mg. and 66.5 mg. per 100 cc. above the initial level, and in 5 patients without anemia there were increases of 60.9 mg. to 100.9 mg. per 100 cc.

The disappearance of the plasma hemoglobin after the termination of hemoglobin infusion was usually rapid, especially during the first four hours. The plasma hemoglobin of all the samples taken 19 hours after infusion was restored to the initial level. The rate of disappearance is roughly proportional to the increase of the plasma hemoglobin level.

Hypothetically, the variation of the plasma volume in the patients would ultimately influence the plasma hemoglobin level in such a study, one having a greater plasma volume giving a relatively high hemoglobin tolerance and vice versa. However, judging from the lack of relation of the body weight (which is a rough index of blood volume) and the amount of the increase in plasma hemoglobin as a result of hemoglobin infusion as shown in Table 1, this factor seems to be an insignificant one.

Moreover, because of the varying severity of anemia in these patients, their blood hemoglobin level variations of from 4.5 gm. to 14.2 gm. per 100 cc., and different levels of hematocrit, the plasma hemoglobin level obtained by hemoglobin infusion may be affected by possible variations of plasma volume. Therefore, the following experiments were performed to determine whether this factor has any influence on the results obtained by this infusion study.

A patient with multiple myeloma who had a hemoglobin of 5.8 gm. per 100 cc. and a hematocrit of 18.2% had the plasma hemoglobin increased by 81.9 mg. per 100 cc. at the end of six hours of infusion (a total of 6.62 gm. of hemoglobin was given). Two days later, after he was given transfusions of 5 pt. of homologous blood (in a period of two days), his hemoglobin level was up to 13.5 gm. per 100 cc. and his hematocrit, to 41.5%. The hemoglobin-infusion experiment was repeated at this time, and the plasma hemoglobin increased by 82.5 mg. per 100 cc. at the end of six hours of hemoglobin infusion. The curves of the hemoglobin tolerance in both experiments are approximately the same, as shown in Figure 2.

None of the patients of the hemolytic group who exhibited increase of plasma hemoglobin level of less than 35 mg. per 100 cc. showed hemoglobinuria. The two patients with paroxysmal nocturnal hemoglobinuria who had perpetual hemoglobinemia displayed marked hemoglobinuria shortly after the termination of the first hour of infusion. Totals of 620.3 mg. and 251.1 mg. of hemoglobin were excreted within a period of 24 hours.

Five patients of the nonhemolytic group exhibited slight hemoglobinuria. One patient with Cooley's trait and an increase in plasma hemoglobin to 49.2 mg. per 100 cc. at the end of five hours of infusion showed slight hemoglobinuria of 44.9 mg., excreted one hour after the end of infusion. The other three patients excreted totals of 27.8, 25.8, and 9.8 mg. of hemoglobin during or shortly after the hemoglobin infusion. There is a marked variation of the renal threshold for hemoglobin excretion among these pa-

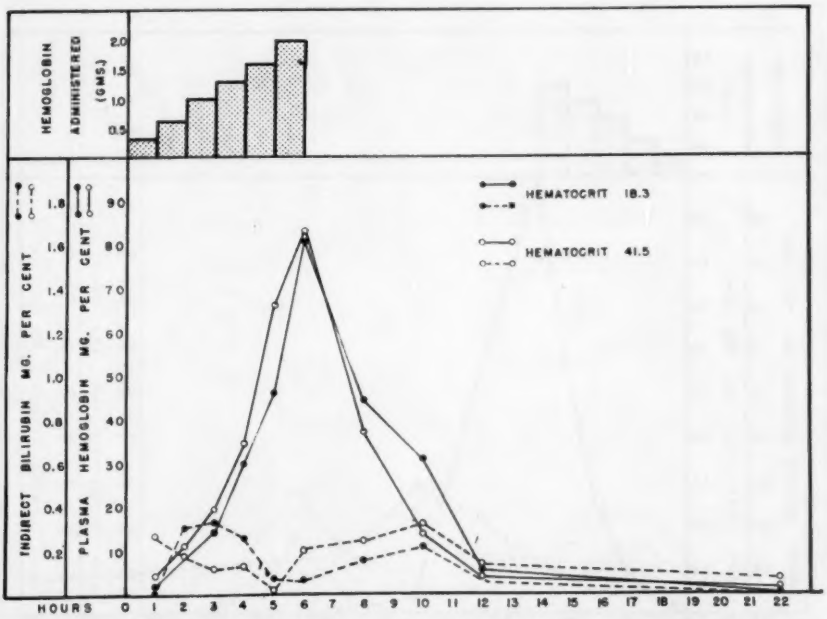

Fig. 2.—Multiple myeloma. Increase in plasma hemoglobin and indirect bilirubin concentration during and following intravenous administration of hemoglobin solution in successive hourly increasing amounts to the same person, hematocrit 18.3% and 41.5% after repeated blood transfusions.

tients. One patient of the nonhemolytic group who exhibited an increase of plasma hemoglobin to 100.9 mg. per 100 cc. at the end of infusion (which is the highest in the series) showed no evidence of hemoglobinuria. Furthermore, those patients who excreted hemoglobin in the urine due to infusion, either during or after, did not necessarily have a high value of plasma hemoglobin.

The urine specimens obtained from the patients who had no evidence of hemoglobinuria due to infusion (with the exception of a transient presence of albuminuria in some of the cases) were all normal. In those patients with hemoglobinuria as a result of hemoglobin infusion, albumin and hemosiderin in urine are invariably present. Hemoglobin casts are found in some of these cases. However, their presence is transient and usually does not last over 24 hours. No patients developed the usual clinical manifestations of lower nephron nephrosis, such as oliguria, anuria, or vomiting.

The plasma indirect bilirubin increased in all the patients, either during or after infusion, but the amount of increase varied greatly from 0.1 to 1.6 mg. per 100 cc. in the hemolytic group and 0.1 to 0.70 mg. per 100 cc. in the nonhemolytic group. The maximum values are usually reached at the end of infusion or two to four hours after infusion. It seems to be greater in patients with liver complication. In seven cases in the hemolytic group with liver involvement, six had values increase by 0.52 to 1.6 mg. per 100 cc. and one had only 0.23 mg. per 100 cc. In two cases with liver metastases in the nonhemolytic group, the plasma indirect bilirubin was increased by 0.62 and 0.70 mg. per 100 cc. It is interesting to note that in the two cases of paroxysmal nocturnal hemoglobinuria who exhibited marked hemoglobinemia and hemoglobinuria after infusion very little increase in plasma indirect bilirubin was found (0.12 and 0.21 mg. per 100 cc).

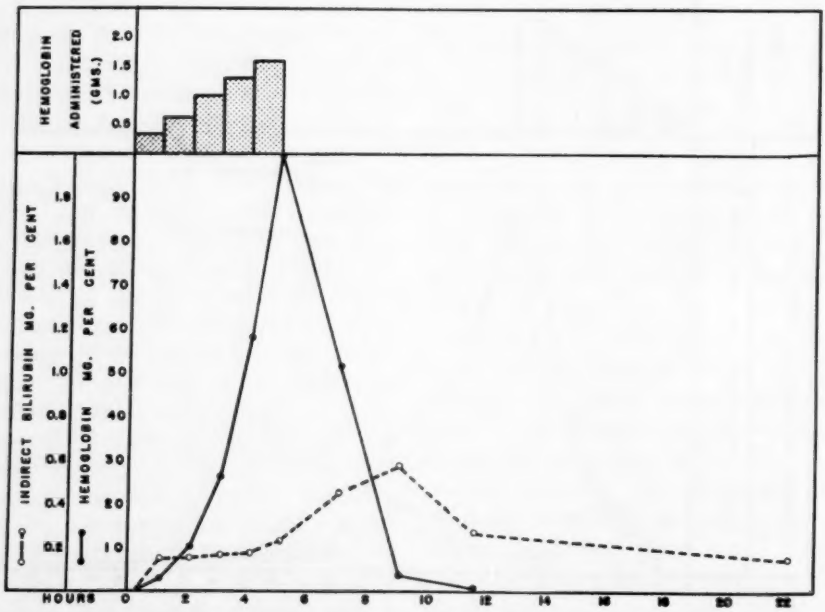

Fig. 3.—Carcinoma of larynx. Increase in plasma hemoglobin and indirect bilirubin concentration during and following the intravenous administration of hemoglobin solution in successive hourly increasing amounts.

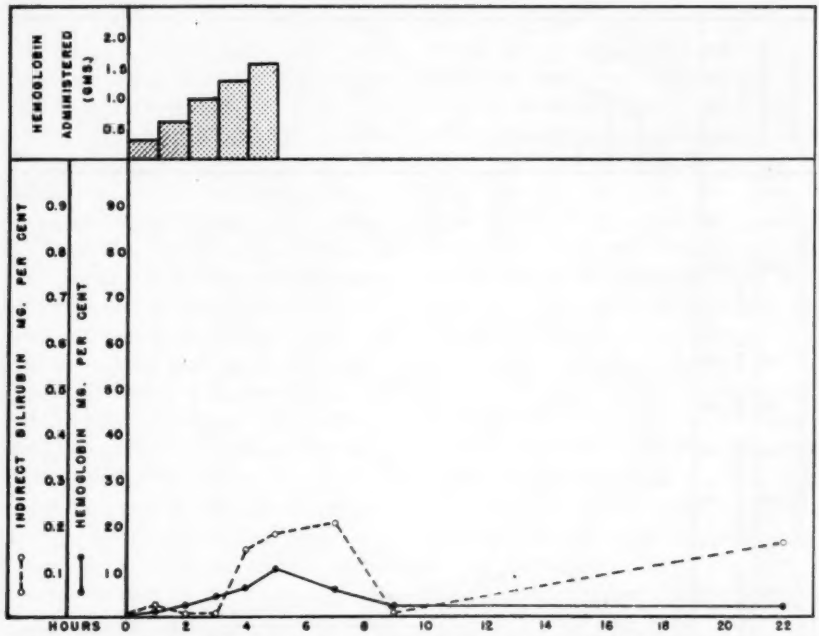

Fig. 4.—Acquired hemolytic anemia. Increase in plasma hemoglobin and indirect bilirubin concentration in plasma during and following the intravenous administration of hemoglobin solution in successive hourly increasing amounts.

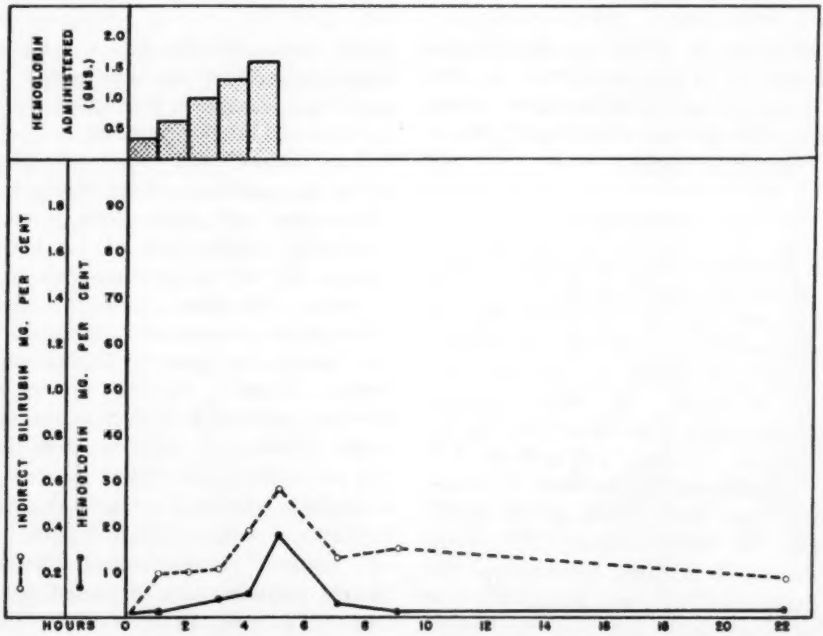

Fig. 5.—Myeloid metaplasia. Increase in plasma hemoglobin and indirect bilirubin concentration during and following the intravenous administration of hemoglobin solution in successively hourly increasing amounts.

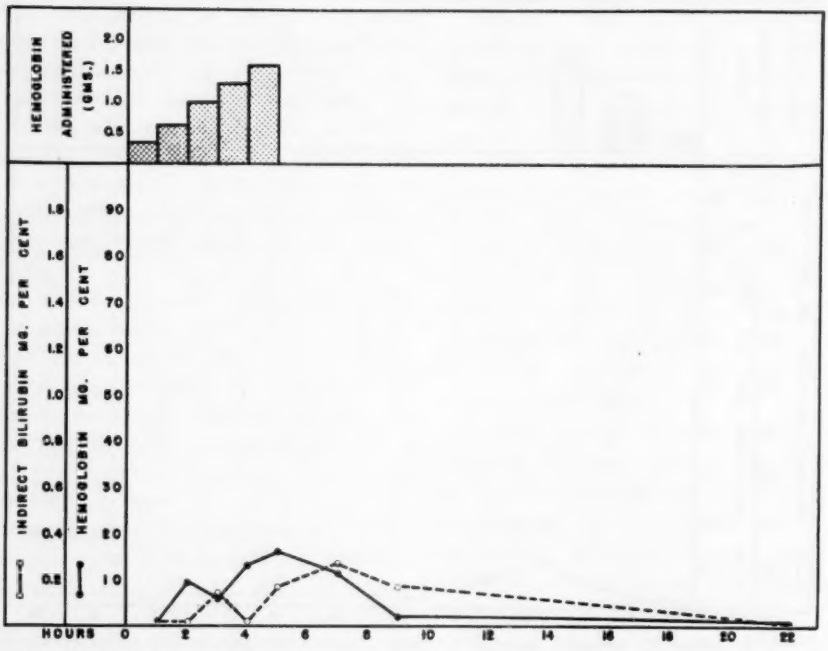

Fig. 6.—Sickle-cell anemia. Increase in plasma hemoglobin and indirect bilirubin concentration during and following the intravenous administration of hemoglobin solution in successive hourly increasing amounts.

Illustrations of the hemoglobin tolerance curve and the increase in indirect bilirubin as a result of hemoglobin infusion in one nonhemolytic and three hemolytic patients are presented in Figures 3 to 6.

Comment

The conception that hemoglobin is transformed into bilirubin is probably very old. Frerichs and Städler,[7] in 1850, observed bilirubin excretion in the urine after injection of bile acid, which is a hemolytic substance. Hermann,[8] in 1859, obtained bilirubinuria after injection of distilled water. Gilligan, Altshule, and Ratevsky,[9] in 1941, demonstrated an increase of plasma bilirubin after the injection of hemoglobin solution. In 1874, Tarchanoff,[10] working with bile-fistula animals, demonstrated that the introduction of pure hemoglobin into the circulation led to increased excretion of bile pigment by the liver. Minkowski and Nauny,[11] in 1886, observed that arsine no longer produced jaundice in geese after hepatectomy. They concluded that bilirubin which is a breakdown product of hemoglobin is found in the epithelial cells of the liver. McNee[12] and Lepehne[13] also observed biliverdin in the Kupffer cells of normal pigeons and geese. Rich,[14] in 1925, found that the removal of the liver decreased the jaundice produced by phenylhydrazine in dogs. Auld[15] observed biliverdin in the endothelial cells of the rabbit's spleen after phenylhydrazine administration. Mann,[16] in 1926, found more bilirubin in the spleen than in the splenic artery. Nisimaru,[17] in 1931, also showed that biliverdin is excreted in the bile when hemoglobin solution is perfused through the frog liver. Eppinger,[18] 1920; Singer,[19] 1945, and Watson,[20] 1937, observed that urobilinogen excretion was decreased by splenectomy, particularly in hemolytic anemia. Mann and his co-workers,[16] London and Kryzanowskaja,[21] have shown that the bone

marrow may also be a site for bile formation. It was not until recently that Jandl et al.[22] demonstrated that Cr^{51}-tagged hemoglobin was retained in the liver and spleen after introduction into the circulation. These studies strongly suggested that hemoglobin break-down was probably situated in the reticuloendothelial cells. According to Laurell's recent paper,[23] plasma normally contains a protein, hepatoglobin, which apparently has a high affinity for hemoglobin, and free hemoglobin will not appear in the plasma until after the hepatoglobin is totally bound with hemoglobin. In cases of hemolytic anemia, an excessive amount of hepatoglobin may be produced by the reticuloendothelial system.

The high hemoglobin tolerance in chronic hemolytic anemia found in this study suggested that the removal of plasma hemoglobin is accelerated in these patients. In consideration of the results of experimental investigation cited above, it appears that this rapid removal of plasma hemoglobin is performed in some way through the reticuloendothelial system.

The amount of hemoglobin found in the plasma after such infusion depends upon the rate of removal of hemoglobin either directly by the tissues (presumably reticuloendothelial cells), or indirectly by conversion of hemoglobin to some other form, or through urinary excretion. It would appear from the observations reported above that absence of hemoglobin in the urine after infusion in these hemolytic cases may be explained by the former mechanisms; also, the low level of plasma hemoglobin excludes the possibility of a renal factor, either a decrease of capacity of glomeruloinfiltration or an increase in renal tubular reabsorption.

The unexpectedly low hemoglobin tolerance with marked hemoglobinuria found in the two cases of paroxysmal nocturnal hemoglobinuria is difficult to interpret. However, in this disease the liver does show central necrosis, which might interfere with the removal of hemoglobin, and the complexity of the factors,[24-26] in the serum influencing the degree of hemolysis of their sensitive erythrocytes may be altered by the infusion of hemoglobin solution thus accelerating hemolysis. Furthermore, since the initial value of the plasma hemoglobin in these patients is high, the free serum hepatoglobin is presumably low, and the hemoglobin tolerance curve will ascend sharply owing to infusion. The intensive siderosis of the convoluted tubules and the loop of Henle without any destruction of the nephron[24] frequently found in this disease may also partly explain the increase in the excretion of hemoglobin in the urine as a result of an alteration of the renal threshold.

According to the recent work based on the survival of compatible transfused red cells in normal recipients, the red blood cells have an average lifetime of about 120 days. Consequently, in the normal person approximately 0.8% of the total number of red cells, containing 7.8 gm. of hemoglobin, is destroyed every 24 hours. With this amount of destruction, the plasma contained only a trace of hemoglobin, approximately 1 to 4 mg. per 100 cc., which is not detectable with the naked eye. However, when the plasma level reaches 10 to 15 mg. per 100 cc., the hemoglobinemia is usually distinct. Therefore, in normal persons the hemoglobinemia is usually detectable if the amount of red blood cell destruction exceeds 2 to 3 times the normal amount. As shown in Table 2, patients of the nonhemolytic group showed an average plasma hemoglobin value of above 10 mg. per 100 cc. at the second hour of infusion (twice the normal destruction), while patients of the hemolytic group reached 10 mg. per 100 cc. (average) between the third and fourth hours of infusion. It is clearly shown that in the hemolytic group, unless the daily destruction of the red blood cells exceeds three to five times the normal amount of destruction, the hemoglobinemia will not be detectable without the aid of a colorimeter for a quantitative determination. This may well explain the absence of gross hemoglobinemia

TABLE 2.—*Plasma Hemoglobin Level at the End of Each Hour Before Successively Increased Rate of Infusion of Hemoglobin Solution*

Hour	Hemoglobin Administered, Gm.	Plasma Hemoglobin, Mg./100 Cc.	
		Nonhemolytic (18 Cases)	Hemolytic (15 Cases) *
0	0	Av. 1.8 (0–5.0)	Av. 1.6 (0.83–4.7)
1	0.33	Av. 3.5 (0.84–7.24)	Av. 1.7 (0.56–3.5)
2	0.65	Av. 10.6 (1.82–17.1)	Av. 4.9 (2.3–7.6)
3	0.98	Av. 20.4 (11.4–34.8)	Av. 9.5 (3.7–14.8)
4	1.30	Av. 34.1 (17.9–70.0)	Av. 14.7 (4.9–22.0)
5	1.63	Av. 57.8 (36.0–103.2)	Av. 22.5 (10.4–34.3)

* Two cases of paroxysmal nocturnal hemoglobinuria and one case of sickle-cell anemia were excluded because of high initial values of plasma hemoglobin.

in chronic hemolytic anemia, even in the presence of a slow intravascular hemolysis.

It is natural to expect an increase of plasma bilirubin following the infusion of hemoglobin solution in these experiments. However, it also depends on the efficiency of the liver in excreting the bilirubin. As shown in the study, the plasma indirect bilirubin increased in all patients after infusion, and it was somewhat greater in patients with liver involvement.

Summary

Eighteen patients with hemolytic processes, thirteen with nonhemolytic anemia of various types, and five without anemia were given by intravenous infusion an isotonic saline hemoglobin solution prepared from human red cells of homologous group. Constant rates of infusion were maintained by an adjustable mechanical pump during successive one-hour periods. All the hemolytic cases showed an increase in plasma hemoglobin below 35 mg. per 100 cc., with the exception of two cases of paroxysmal nocturnal hemoglobinuria. The patients of the nonhemolytic group and those without anemia showed increases above 35 mg. per 100 cc.

Because of the high hemoglobin tolerance in these chronic hemolytic cases, the rate of destruction of their red blood cells will be three to five times the normal before hemoglobinemia is noted. This may explain the clinical observations of absence of hemoglobinemia in these chronic hemolytic cases.

The amount of hemoglobin solution injected did not produce overt symptoms of any kind except transient albumin and hemosiderin in the urine.

The plasma indirect bilirubin value increased in all patients after infusion. It was somewhat greater in patients with liver involvement.

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The Anemia of Renal Failure

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The association of anemia with uremia is a striking one. However, just as the actual pathogenesis of the signs and symptoms associated with renal disease is poorly understood, so also is the mechanism of development of anemia in this syndrome. It is the purpose of these investigations to evaluate the relative importance of productive and destructive defects and to correlate them with the clinical state.

Methods and Materials

Hemoglobin was measured according to Crosby et al.[1] Reticulocytes were counted by the dry method and expressed as per cent of the 1000 cells counted. Osmotic fragility was measured according to the technique of Shen [2] and increment curves plotted and compared as previously described. Mechanical fragility was measured by a modification of the method of Shen et al.,[4] with use of rotation of 35 rpm and a radius of 13 cm. Time intervals varied from 90 to 270 minutes. Ashby survival studies were done using dry anti-M serum or liquid hyperimmune anti-A. The dilutions used were those which provided adequate numbers of cells for valid counting, and the same vial was used throughout a given experiment. Agglutination technique used was that appropriate for the particular antiserum, and blank counts were in a range below 20,000 cells per cubic millimeter when the whole blood final dilution was 1:200.

When Cr^{51} was used for autotransfusion survival studies, 50 ml. of blood was withdrawn from the patient and placed in 12.5 ml. of A. C. D. solution to which 100 to 150μc. of $Na_2Cr^{51}O_4$ had been added immediately before. The total chromate added never exceeded 0.030 mg., which is well below the concentration known to be traumatic to the red cell. After incubation at room temperature for approximately 30 minutes, the tagged blood was reinjected into the patient and samples drawn at intervals thereafter. To avoid variations due to in vivo tagging of red cells, the five-hour sample was the first one used in calculating survival data. The whole blood cell counts were corrected for plasma protein tagging as long as radioactivity could be detected in the plasma. This was always negligible by the fifth day. Curves of normals demonstrated a curvilinear disappearance with a $\tau\frac{1}{2}$ between 27 and 33 days, as has been reported by others. Rather than correct for chromium elution in an abnormal cell population, the results of the studies in uremics are expressed in terms of comparable $\tau\frac{1}{2}$, since one cannot be certain that the elution rate is constant in all states.

Iron turnover studies were done with use of $Fe^{59}Cl_3$ and Fe^{59} citrate as radioactive tags. These were obtained from Abbott Laboratories and when received had a specific activity of 1μc. per 0.5μg. of elemental iron. Normal plasma of known iron-binding capacity was used as the vehicle for the tracer; 8μc. to 10μc. was added to approximately 12 ml. of heparinized plasma, and exactly 10 ml. of this was injected after incubating at room temperature for approximately 30 minutes. The dose used never exceeded half the free iron-binding capacity of the plasma. Samples of blood were drawn at short intervals for the first 80 minutes and then at hourly intervals for 4 to 5 hours. The plasma was separated and 3 ml. counted in a well-type scintillation counter (Tracerlab) with a 3 in. lead shield and a background count between 90 and 100 counts per minute. The half disappearance time was calculated from a semilog plot in the usual manner. The remaining tagged donor plasma was diluted 1:500 and also counted to determine the total amount injected. Thereafter whole blood samples were drawn daily and the radioactivity measured by the same devices. This was done until a plateau was reached. When possible, the study was carried on for six to eight weeks. Body monitoring was performed during the first week in the majority of these cases with a Tracerlab Monitor equipped with a Geiger-Mueller probe. Although the efficiency of this apparatus is very low for surface scanning and does not allow quantitative interpretations, it was possible to determine with some validity the "direction of flow" of the

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iron pools. The end of the probe was held flush against the marked site, and readings were taken by averaging the needle fluctuations. They were expressed as the average counts per minute read from the scale at 11 seconds' integration. The sites counted were the precordium, liver, spleen, sacrum, and thigh. The results each day for a given site were expressed as percentage of the total counts from the five areas, after background values were eliminated. With such a ratio, one could compare one day's counts with the next and evaluate entrance and exit of iron to and from these compartments.

To calculate plasma iron turnovers, plasma volume was measured with Evans blue dye (T-1824). This was injected immediately before the Fe^{59} plasma and 10-minute and 20-minute samples used for measurement of concentration of dye. Plasma iron and iron-binding capacity were measured by the method of Peters et al.[5,6] The normal values for the method in this laboratory are 136μg. per 100 cc. with 1 S. D. of 32μg. for serum iron and 378μg. per 100 cc. with 1 S. D. of 53μg. for serum iron-binding capacity. Plasma iron turnover per milliliter of whole blood allows comparison with normal subjects without reference to variations in body mass and blood volume. It is also expressed per milliliter of red cells in order to compare this value with circulating red cell volume without regard to anemia.

Turnover is determined by multiplying the plateau fraction of isotope appearing in the red cells by the total plasma iron turnover. This is not an absolute measure of the uptake from a single turnover, since iron is incorporated throughout the development of the red cell and since recirculation between depots may occur and lead to falsely high values. However, the plateau is the most accurate point to assess on the uptake curve and does give data for comparison. It is obvious that the values of normals are higher than the theoretical calculations would allow. The red cell iron turnover is expressed per milliliter of red cells to relate it to the actual red cell volume as well as per milliliter of whole blood to compare results with those in the normal nonanemic subject.

The two normal subjects fell within the range of values reported by Giblett et al.[7] With use of figures from these authors and modification of them to expressions per milliliter of red cells and per milliliter of whole blood, assuming a venous hematocrit of 45%, the limits of normal have been calculated and are included in the Table.

The patients studied were taken from the wards of the Boston City Hospital. The age span was from 17 to 93, with a predominance in the older age group. The clinical diagnoses were as follows: chronic pyelonephritis, 25; nephrosclerosis, 7; chronic glomerulonephritis, 5; gout, 1; acute renal shutdown, 1; chronic renal disease of undetermined etiology, 15. The population was selected on the basis of evidence of renal failure by NPN, BUN, PSP, or unexplained anemia in the presence of chronic renal disease. Hematocrit varied from 13% to 49%, and red cells were normochromic in all cases. In some patients, anemia increased during the studies. In no case did it improve.

Results

To investigate the possibility of abnormalities of the circulating red cells, mechanical and osmotic fragility tests were done. In Figure 1 the results have been plotted; they demonstrate an increase in mechanical fragility in 20 of 47 tests in 40 patients. There were six patients in whom more than one study was done. One had two normal

Values for Fe^{59} Turnover Studies *

Case	Serum Iron, μg./100Cc.	T ½, Min.	Plasma Iron Turnover/Day			Red Cell Iron Turnover/Day		
			Total, Mg.	Per Ml. RBC, μg.	Per Ml. WB, μg.	Total, Mg.	Per Ml. RBC, μg.	Per Ml. WB, μg.
18	36	28	55	49.0	12.2	55	49.0	12.2
26	119	32	198	166.0	33.3	198	166.0	33.3
27	27	73	10.5	13.0	2.88	10.5	13.0	2.88
28	120	112	43	26.9	7.59	43	26.9	7.59
29	66	80	25.7	18.5	5.84	20.5	14.7	5.01
30	222	254	37.3	29.15	6.72	12.2	9.5	2.38
31	28	42	29.7	28.0	5.4			
32	31	29	20.8	30.5	7.75	20.8	30.5	7.75
33	65	56	64.4	28.0	8.2	64.4	28.0	8.2
34	33.5	52	20.3	19.8	4.8	15.1	14.7	3.6
35	145	113	52.5	32.0	10.1	32	19.5	6.2
36	128	256	16.5	14.4	3.7	1.0	0.88	0.25
37	19	18	33.4	36.9	8.2			
38	32	25.5	30	39.8	9.6	30.0	39.8	9.65
39	12.5	32	9.25	9.65	2.8	8.05	8.4	2.7
40	11.5	32	9.92	7.9	3.3	7.65	6.1	2.52
Normal range								
Upper	200		42.7	24.35	10.95		17.60	7.93
Lower	72		27.5	16.6	7.77		12.48	5.75

* Tabulated for the individual patients ,with the normal range for each value given for comparison.

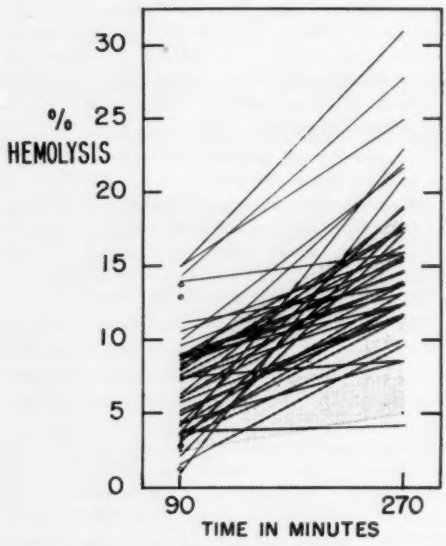

Fig. 1.—The mechanical fragility, expressed as per cent hemolysis, is plotted according to values observed at 90 minutes and again at 270 minutes. The shaded area represents the normal limits.

and two high values; another had two tests done and both were abnormal; the remaining four each had one normal and one abnormal result.

There was no correlation between increased mechanical fragility of red cells and N. P. N. or hematocrit. Renal infection was equally common in the cases with normal and with abnormal red cell fragility. Neither was there any clinical criterion which separated the two groups. Attempts to transfer this fragility to normal cells in vitro were unsuccessful.

In 10 patients, the degree of autohemolysis was observed. In none of these was any abnormality noted.

Osmotic fragility curves of red cells of these patients had normal shapes with a single population type of distribution. There were 2 of 28 blood samples which demonstrated 1% hemolysis in 0.54% saline. All other samples were completely free of hemolysis until concentrations of 0.50% NaCl or less were reached. There was a single shift of the curves to the right in 11 of 28 instances, with the major mode at 0.30% NaCl rather than within the range of 0.34% to 0.38%. Incubated fragility results were not remarkable. There was no correlation between the presence of this slightly resistant population and anemia, azotemia, or increased mechanical fragility.

Thus, while abnormalities of fragility could be demonstrated in a number of these patients, the deviation was usually not great, nor was there correlation evident between this and other laboratory or clinical findings.

To evaluate whether these red cells were undergoing premature destruction in vivo, red cell survival studies were observed in 14 patients with use of Cr^{51} and in three with the Ashby technique. The autotransfusion (Cr^{51}) curves, plotted in Figure 2, demonstrate normal $\tau\frac{1}{2}$ valves in only three cases. One of these was a patient who was convalescing after an episode of acute renal shutdown; another was a patient with chronic renal disease whose N. P. N. had returned from elevated to normal levels in the hospital with anemia persisting; the remaining case had chronic renal disease with mild azotemia and chronic anemia. Two patients died of renal failure during the course of this experiment, and it is interesting to note that the rate of hemolysis did not differ strikingly from that of surviving patients. It is evident from this figure that moderate hemolysis is a common lesion in this patient population.

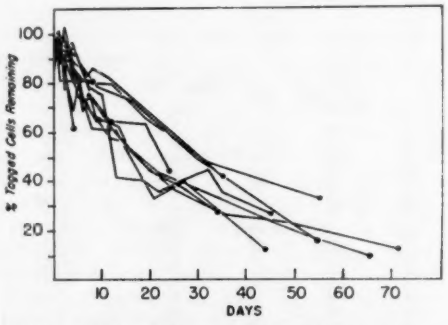

Fig. 2.—The values of Cr^{51}-tagged red cell survival studies are plotted with the 100% value derived by extrapolation to zero time. Time in days is plotted against per cent of tagged cells remaining in the circulation.

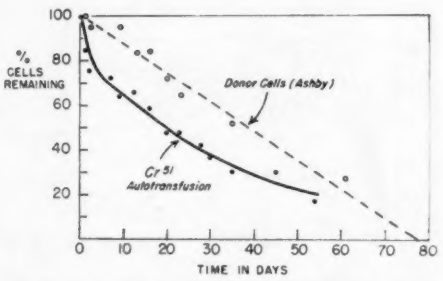

Fig. 3.—A simultaneous study of the survival of patient's cells and donor cells is charted. Time in days is plotted against the per cent of the indicator red cells in the circulation. The broken line delineates the curve of donor cells followed by Ashby technique. The solid line is the survival curve of the Cr^{51}-tagged autotransfused cells.

To determine whether the hemolytic defect is extracorpuscular, simultaneous studies were done in two patients by tagging the patient's own cells with Cr^{51} and measuring survival of donor cells simultaneously with the Ashby technique. In Figure 3, in which one of the studies has been plotted, it is obvious that there is hemolysis of normal donor cells as well as the patient's own. In one patient, Ashby survival studies done alone also demonstrated a mild hemolytic defect with a red cell life of 75 days.

Thus, a hemolytic factor of extracorpuscular type was commonly demonstrated in these patients. There was no correlation between the rate of hemolysis and the hematocrit, nor did the decreased red cell survival time appear to correlate with azotemia; in one case this lesion was demonstrated three months before elevation of the N. P. N. was documented. Because the patient population studied did not allow a reliable measure of the duration of renal disease and renal failure in many cases, it could not be determined whether these elements correlated with the degree of hemolysis.

It is obvious that the rate of hemolysis would not have led to anemia in these patients were the normal compensatory process of the erythropoietic tissue available. Therefore, in another group of patients, investigation of iron turnover was carried out in order to evaluate the productive defect in this disease. The patients had varying renal disease, with the common denominator being chronic renal failure. In the Table, the ferrokinetic indices have been tabulated. The diversity of results is evident and demonstrates the multiplicity of defects in this condition.

In this group of patients and in an additional 23 cases, serum iron was measured, and in 26 it was found to be low, the average value being 67.6μg. per 100 cc., with a standard deviation of 45 μg. Eliminating a value of 222μg. per 100 cc., the values were 63.5μg. per 100 cc., with a standard deviation of 39μg. The total iron-binding capacity was low in 9 of 36, with an average of 321μg. per 100 cc. and a standard deviation of 59 μg. In only two of the cases with decreased serum iron was iron deficiency demonstrated by a suboptimal response to iron therapy. In the remainder, one must attribute the low value to redistribution of iron pools. In one case, in whom there had been multiple episodes of gastrointestinal bleeding in the recent past, the finding of a normal serum iron value was somewhat surprising. However, this patient had markedly diminished red cell production demonstrated by ferrokinetic indices, and it is possible that the lack of iron utilization allowed a normal serum iron level even in the presence of diminished iron reserves. The same sort of erythropoietic defect in another patient, in whom there had been no prior loss of iron, led to an elevated serum iron. Thus the prior stores and varying demands played some part in regulating the serum iron level, but the pattern was in general a low value associated with low or normal total iron-binding capacity.

The disappearance curves of the tagged plasma iron appeared as straight lines on semilog paper in most cases, with a possible tendency for a more complex slope in a few instances where the disappearance was rapid. In the latter group, a straight line was drawn through the main slope and the half-time calculated from it. The wide variation

in $\tau\frac{1}{2}$ values is seen in the Table. This function depends on serum iron level as well as marrow demands, and therefore the actual turnover values are more useful indices.

The plasma iron turnover per milliliter of whole blood was abnormally high in 2 of 16 cases, normal in 4, and decreased in 10. This index is considered to reflect erythropoietic activity [8] and by these figures 10 of 16 cases demonstrated less active erythropoiesis than the nonanemic controls. All these patients were anemic, and in such an instance one would expect an increased response on the part of the marrow. Expressed per milliliter of red cells, the plasma iron turnover thus reflects the erythropoietic activity required to replace this volume of cells and demonstrates that in most cases the plasma iron turnover per unit cell is actually increased in spite of the fact that the rate per milliliter of whole blood is normal or less. This is consistent with the decreased survival time of red cells in this syndrome.

The patients with marked increased turnover demonstrate the lack of uniformity of pathogenic mechanisms in this disease. Case 26 was extremely ill with terminal chronic glomerulonephritis and showed evidence of brisk hemolysis as well as a marked erythroid shift of marrow cells. Case 18 was a patient who had iron deficiency superimposed on the anemia of renal failure due to chronic pyelonephritis. The four patients with the low turnover values per milliliter of red cells had pyelonephritis and nephrosclerosis of varying severity. One could not correlate these values with clinical status.

Red cell iron turnover should be a more direct means of measuring red cell production. These calculations demonstrate the same trends and suggest that there is a significant productive defect in many of these cases.

The iron reappearance curves demonstrate both percentage utilization and rate of appearance of the iron. There is a tendency for the cases with the greatest utilization to have the most rapid increases to plateau

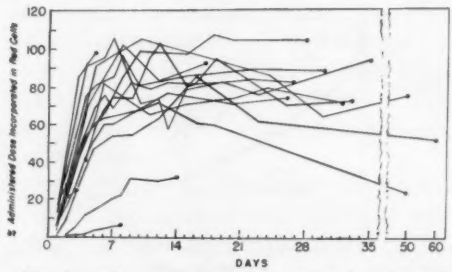

Fig. 4.—The percentage of the total dose of Fe^{59} incorporated in the circulating red cells is plotted according to the number of days after administration of the isotope.

in these curves (Fig. 4). The curves showing less utilization have more gradual slopes and probably reflect erythropoiesis over a longer time span, leading to falsely high values. Again these observations suggest that a number of these patients have marrow hypofunction.

It is interesting to note the pathway of this iron by surface monitoring. In the patients in whom red blood cell iron utilization was within normal limits, the surface studies demonstrated the normal directional flow from plasma to marrow to blood without evidence of ectopic erythropoiesis or early hemolysis. In those in whom turnover values were low surface scanning revealed a high percentage of the radioactivity to be found over the liver area and with little associated "splenic" activity. Their sacral activity was evident but small. In three patients who died while under observation with Fe^{59} studies, tissue samples were counted and confirmed the observations of surface scanning in the individual cases.

An example of a patient with active erythropoiesis is demonstrated in Figure 5. The expected transient activity over the sacrum is clearly evident. Also in Figure 5 is a chart of the radioactivity curves in a patient with decreased turnover. In this case, the major curve is over the liver area without subsequent discharge, demonstrating iron deposition in storage by default of the marrow.

In summary, the majority of these patients demonstrate marrow insufficiency of

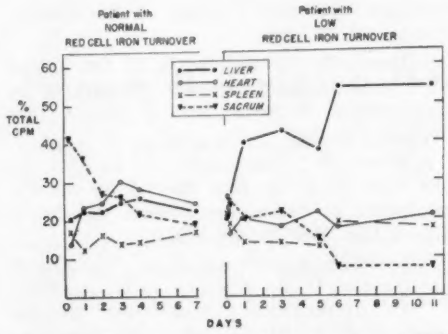

Fig. 5.—The results of surface scanning of Fe^{59} in two uremic patients are plotted for comparison. The surface radioactivity is expressed as a per cent of the total counts noted over the heart, spleen, liver, sacrum, and thigh after corrections for background. Thigh has not been included in the chart, since the activity was constantly low. The values are plotted against the day of observation.

varying degrees to a point where erythropoiesis is depressed below the ratio of a normal nonanemic subject in some patients. There was no correlation between the depression of erythropoiesis and the degree or duration of renal failure, the type of renal disease, urinary findings, or the hematocrit in the group of patients studied. The fact that some patients did have normal or increased values emphasizes that this is an anemia of multiple defects.

Comment

Although anemia is a constant complication of renal failure, the mechanism of its development is obscure. That the degree correlates with the degree of azotemia has been suggested [9,10] but this relationship has such wide variation that it is of little significance in the individual case. It is evident that anemia is, itself, a manifestation of renal failure, irrespective of the type of underlying renal disease.

In a situation where anemia is such a constant finding, it is interesting that it cannot be attributed to a single defect, but rather results from several pathogenic mechanisms. That there is a hemolytic element has been suggested by several workers,[11-13] and in the present series of patients this defect was a very common one. By comparing the survival of donor cells with autotransfused blood, it could be demonstrated that an "extracorpuscular" factor was responsible for the hemolysis. The tendency toward abnormal fragility of the red cells suggests that this factor has had some effect, direct or indirect, on the red cells prior to hemolysis.

The degree of abnormality noted in these patients cannot be explained by a distorted age distribution of the red cell population. Evidence has been cited in the past to suggest that these fragile cells are the result of a hemolytic mechanism.[14] The in vitro "storage lesion" is associated with increased mechanical fragility and is reversible when the cell is reintroduced into a normal environment.[15] Studies recently reported [16] suggest that in uremia there is a red cell defect of similar nature with a diminished reserve of high-energy phosphate, and the increased fragility may be reflecting this chemical defect. Such a situation in vivo may well be associated with shortened red cell survival, since the integrity of the red cell depends on glycolysis.

Hemolysis of the degree noted in most of these patients is a nonspecific manifestation of disease and has been described in infection, malignancy, arthritis, and lymphoma. However, the ferrokinetic studies demonstrate the presence of an erythropoietic defect in these patients of a degree not found in other chronic disease. The observations here demonstrate that this marrow insufficiency is unrelated to severity of renal disease, type of disease, or degree azotemia.

One can only postulate the basis for this until methods are available for quantitating erythropoietin in such cases. However, the fact that Jacobson [17] has found a source of this marrow stimulant to be the kidney may have some bearing. Recent work by Erslev [18] demonstrates that uremic rabbits not only fail to respond to erythropoietic stimulus to a normal degree but also have a diminished level of activity in their plasma. The lack of effect of N. P. N. in depressing marrow reported by this same investigator is

consistent with the clinical evidence that this criterion of renal failure is not closely related to any of the pathogenic mechanisms found in these patients. However, with the evidence at hand, it is as yet impossible to determine whether the kidney is responsible indirectly by failing to excrete a toxic product or directly by failing to produce an erythropoietic substance. It is not unlikely that both play some part.

Summary

A group of patients with renal failure were studied to evaluate the pathogenesis of the anemia of uremia.

Hemolysis was found to be a very common abnormality in these patients, but was of only moderate degree. The inability of the marrow to compensate for this was demonstrated by the diminished Fe^{59} turnover indices in these patients.

There was no evident correlation between the predominant pathogenic mechanism and other clinical or laboratory findings in these patients.

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Boston City Hospital (18).

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Primary Reticulum-Cell Sarcoma of the Leptomeninges

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Earlier authors frequently described any rapidly growing, highly cellular intracranial tumor as a "sarcoma." Inadequate clinical and microscopic descriptions, doubtful photomicrographs, the failure to make proper use of special stains, incomplete postmortem examinations, and, especially, the use of multiple synonyms for the same type of tumor makes it very difficult to review the pertinent literature on this subject. More recent discussions are in agreement on one point, however, which is that if one restricts the use of the term "sarcoma" to malignant mesodermal tumors they are very uncommon indeed intracranially. Thus, they made up only 0.7% of 2023 brain tumors in Cushing's [1] series and less than 1% of Olivecrona's [2] even larger series of 4188 cerebral neoplasms.

True cerebral sarcomas occur usually as focal tumor masses (isolated or multiple) with or without local involvement of the overlying meninges. They may also begin in the periadventitial tissue of the leptomeninges and spread diffusely within the subarachnoid space without direct invasion of brain or cord and without distant (extracerebral) metastasis.

The following case report is an example of the rare occurrence of primary intracranial reticulum-cell sarcoma masquerading as a case of chronic meningitis, presumptively tuberculous.

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Report of a Case

A 62-year-old white widowed right-handed "wire-inspector" worked up to two weeks prior to his admission to an outlying community hospital, on Nov. 14, 1954.

Little of his past history is known other than that he had been eating erratically and drinking heavily since the death of his wife some six months previously.

On admission, he was complaining of frontal headaches, insomnia, and bilateral leg pains, but the immediate reason for hospitalization was an acute episode of "confusion." He was found to have "severe hypertension" without papilledema and a mixed type of aphasia. A diagnosis of "hypertensive encephalopathy" was made; spinal puncture was omitted. There was no evidence of skin eruption, generalized lymphadenopathy, or enlargement of liver or spleen.

A week after admission he became increasingly agitated, tremulous, and noisy, and he was transferred to a psychiatric institution on Nov. 27, 1954.

There he was reported to be disoriented, euphoric, and suspicious. He wandered about aimlessly, complained of severe headache, but had no convincing focal neurologic signs. Four days later, however, his neck was stiff and early papilledema appeared bilaterally. After spinal puncture (Table)

Cerebrospinal Fluid Findings

No.	Date	Pressure, Mm. Water		Color	Total WBC	PMN Cells, %	Protein, Mg./100 Ml.	Sugar, Mg./100 Ml.	Cultures
		Initial	Final						
1	12/1/54	480	200	"Green"	900	80	218	40	Negative
2	12/16/54	400	170	Cloudy yellow	1,100	70	465	69	Negative
3	12/23/54	450	120	Cloudy yellow	950	5	420	68	Negative
4	1/10/55	480	480	Cloudy yellow	1,800	4	335	85	Negative

he was transferred to a hospital for infectious diseases as a "meningitis suspect." Shortly thereafter (Dec. 3, 1954) he was transferred to the Boston City Hospital Neurosurgical Service as a "brain-abscess suspect." When a ventriculogram showed symmetrically dilated lateral ventricles, with the third and fourth ventricles well-visualized, he was transferred to the Neurological Service.

Up to this point, his temperature and vital signs (including blood pressure) had remained within normal limits. He showed evidence of recent weight loss, but he had no lymphadenopathy or enlargement of liver or spleen. He was confused, markedly disoriented, and tremulous, and he had vivid hallucinations in which he conversed with the Devil. His neck was semistiff. The optic discs were blurred, with hemorrhages in the right fundus. There was bilateral sixth-nerve involvement and right central facial paresis. The right arm tended to "drift" downward when held extended with eyes closed. Sensation to pin-prick seemed normal. There was a right extensor plantar response.

Laboratory Studies.—Hemoglobin was 13 gm.; white blood cell count was 18,000, with 70% to 80% polymorphonuclear cells. Urine was normal. The blood Hinton test was negative, as were the serologic tests for syphilis on the spinal fluid. Blood cultures and examination of bone marrow were negative.

X-Ray Studies.—Metastatic series including chest and skull films were negative. Paget's disease of the left femur was found.

Course.—Although repeated bacteriologic and fungus studies on the spinal fluid were negative, the patient received intensive antituberculous therapy consisting of streptomycin, isoniazid, and aminosalicylic acid, on the assumption that tuberculous meningitis was the only treatable disease consistent with this constellation of clinical events.

The findings on serial spinal puncture are summarized in the Table.

He gradually lapsed into a semicoma and died on Jan. 24, 1955, about 12 weeks after the onset of neurologic symptoms.

Postmortem Findings.—General postmortem findings were consistent with hypertensive cardiovascular disease. There was no systemic evidence of carcinoma, tuberculosis, syphilis, generalized Paget's disease, or lymphoma.

The brain was examined after fixation in 10% formalin.

Gross Findings: Dura and sagittal sinus showed no abnormality. The cerebral hemispheres were markedly swollen, right more than left, with herniation of swollen and locally infarcted brain through the bur holes. There were, however, no subfalcial, uncal, or tonsillar herniations.

Although no discrete nodules or tubercles could be seen, there was a generalized milky appearance to the leptomeninges over both hemispheres, especially about the pial veins.

On sectioning the brain coronally, the most striking lesions were seen in the subarachnoid space and adjacent cerebral cortex (Fig. 1). No discrete lesions were seen in the cerebellum, brain stem, or basal ganglia.

The involved areas showed a gelatinous, glairy coagulum, varyingly green or yellow. The subarachnoid spaces, especially at the depth of the sulci, were distended by this material. The outer layers of cortex were discolored but, in the gross, not invaded by the material seen in the subarachnoid

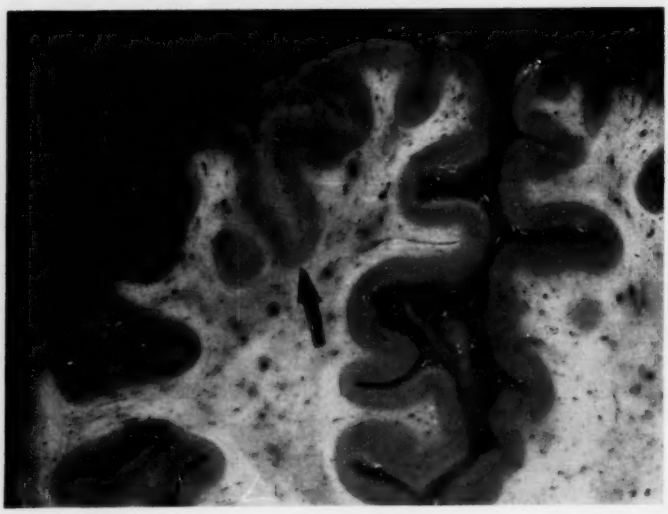

Fig. 1.—Right frontal lobe. A glairy mass of tumor is distributed in the subarachnoid space over the convexity, especially in the depths of the sulci. Although some areas appear free of tumor, no part of the subarachnoid space was free of microscopic tumor.

Fig. 2.—Right parietal lobe. The tumor cells fill the subarachnoid space but do not invade parenchyma of brain except along the perivascular spaces. Only the adventitia of arteries is infiltrated. Hematoxylin and eosin.

space. The lateral ventricles were mildly dilated but showed no ependymal lesions.

At this point, the provisional anatomic diagnosis was "Cryptococcus (neoformans) meningitis."

Microscopic Findings: Multiple sections of the spinal cord, brain stem, cerebellum, and cerebrum were taken.

There was intense and diffuse infiltration of the subarachnoid spaces by sheets of tumor cells (Fig. 2). These were of uniform size, 12μ to 14μ in diameter, with abundant reticulum and scanty cytoplasm; nucleoli were not prominent. They were considered typical of reticulum-cell sarcoma (Fig. 3).

In most areas these cells had not invaded brain. An exception to this was in the infundibular stalk, which was almost replaced by tumor cells. In the rest of the brain and cord there was extensive infiltration from the subarachnoid space along the perivascular spaces. Practically all the emergent cranial and spinal nerves were surrounded and infiltrated with tumor cells (Fig. 4).

All of the blood vessels near and in the subarachnoid space were surrounded by tumor cells. The tumor involved only the adventitia of arteries, but in the veins there appeared to be complete replacement of the vessel wall by tumor, without thrombosis.

The subependymal veins were likewise heavily infiltrated, but no accumulation of tumor was seen on any ependymal surface.

Many hemosiderin-filled macrophages were seen in the small necrotic areas of corpus callosum. This change was presumed to be secondary to the effect of ventricular puncture.

Comment

The designation "reticulum-cell sarcoma" in this case appears justified to us according to the criteria set up by Jackson and Parker [3] and by Sparling et al.[4] In a recent excellent review of the neurologic manifestations of the lymphomas, Whisnant [5] and the Mayo Clinic group found 8 cases of reticulum-cell sarcoma with invasion of the central nervous system in a total group of 76 patients with lymphoma. Only three of the eight tumors were intracranial, and none involved the meninges primarily. By far, the commonest presenting symptom in such cases is severe back or extremity pain.

It is difficult, for the reasons stated before, to compare our case showing meningeal invasion only with other neoplasms reported in the literature descriptively as "perivascular sarcomas," [6-8] "perithelial sarcomas," [9-11] "alveolar sarcomas," [9,10] and "microgliomas." [12]

The following histologic features were most impressive in our case: (*a*) marked perivascular and perineural infiltration by sheets of tumor cells, (*b*) dense reticulum about blood vessels and encircling tumor cells, (*c*) invasion of the walls of venules without thrombosis, (*d*) no systemic evi-

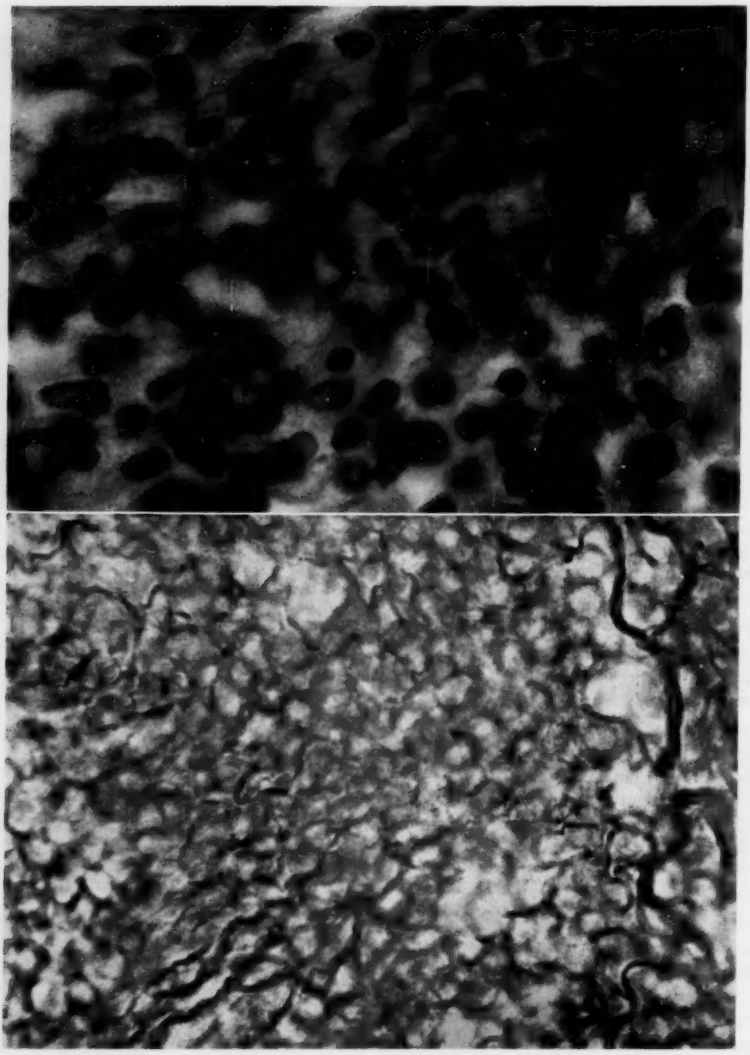

Fig. 3.—Top, the cells are of more or less uniform size, with scanty cytoplasm and rather densely staining nuclei. Hematoxylin and eosin; reduced about 23% from mag. × 800. Bottom, there is abundant reticulum, in most places surrounding even individual cells. Laidlaw's reticulum stain; reduced about 23% from mag. × 800.

dence of lymphomatous disease, and (*e*) dura not involved.

Kinney and Adams,[13] in their comprehensive review of primary reticulum-cell sarcoma of the brain in 1943, reported two cases and accepted six others from the literature. All of these occurred in males over 35 years of age and in the temporal lobes of the brain. In none of these were the leptomeninges grossly involved. This focal type of involvement has been reported since,[14,15] but the lesions have not been confined to the temporal lobe.

There are less than a half-dozen cases recorded resembling our own and simulating chronic meningitis rather than single or multiple focal lesions[16-18] and one case[19] in which the clinical picture was that of

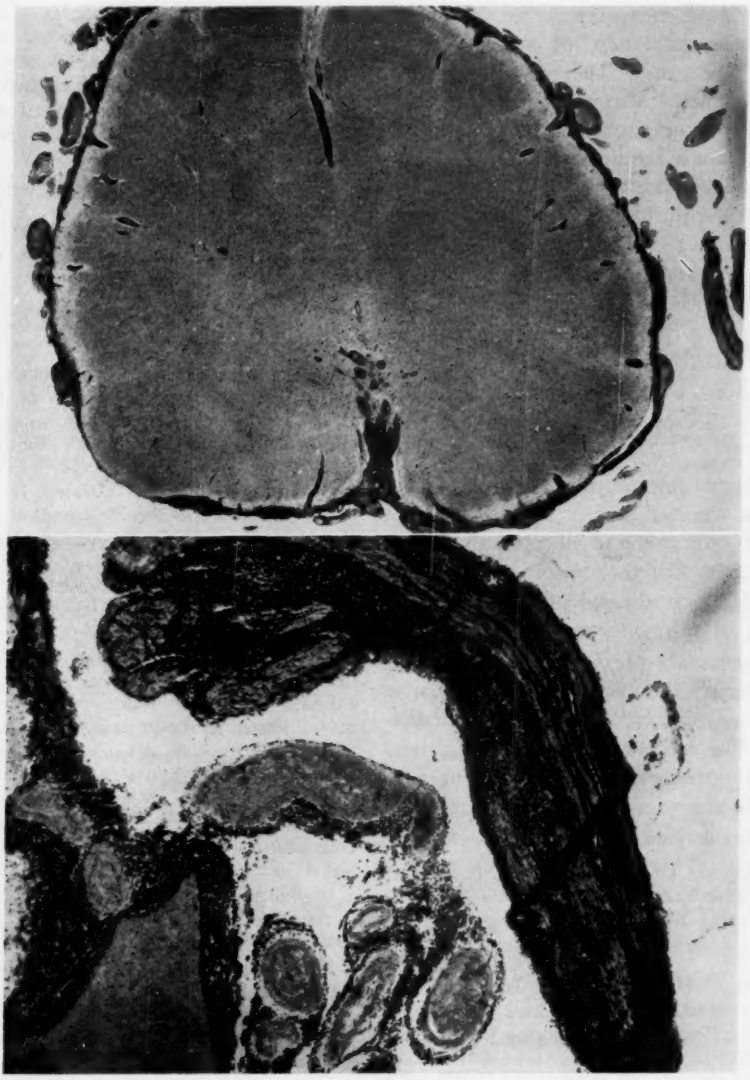

Fig. 4.—Top, the subarachnoid space about the medulla is filled with tumor, and there is tumor infiltration of the nerve roots. Bottom, a higher power view of one of the roots of the eleventh cranial nerve. All the cranial and spinal roots were infiltrated to a degree.

acute "infective" polyneuritis. Clinically, headache and progressive dementia have been prominent in these cases, but the clinical picture is otherwise nonspecific. In retrospect, it might have been possible to establish the diagnosis in our case before death by repeated careful examination of cell blocks of centrifuged spinal fluid with appropriate stains. Moossy and Sorrell [17] were able to arrive at a tentative diagnosis of "malignant lymphoma" in their case by taking frontal and parietal brain biopsy specimens at craniotomy. Vigorous radiotherapy through large fields was instituted, but death occurred in four months.

The over-all evidence seems to us to establish this process as a diffuse leptomeningeal invasion by reticulum cells (de-

rived from mesenchyme) rather than as a neoplastic degeneration of microglia, as Russell [12] believes. The clinical picture closely simulates chronic meningitis, but studies for fungi, tuberculosis, and metastatic carcinoma are negative. The cerebrospinal fluid, because of a polymorphonuclear pleocytosis with high protein but normal sugar and negative smears and cultures, suggests the presence of intracranial sepsis or a granulomatous or mycotic process. Because of the rarity of this condition and the difficulty in diagnosis, the reported results of radiation or mechlorethamine (nitrogen mustard) therapy are too few to evaluate critically.[20]

The special interest of this case (and the very few reported similar to it) lies in the remarkable activation of all connective tissue histiocytes within the central nervous system as well as in the endoneurium and perineurium of the spinal and cranial nerves. Unlike the cases of Kinney and Adams [13] and Russell,[12] the microglia do not seem to be primarily involved either focally or diffusely. The stimulus for the diffuse activation of reticulocytes within the nervous system in this case is as obscure as it is in most other instances of neoplasia.

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Thyroid Function and the Metabolism of Iodine in Patients with Subacute Thyroiditis

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Recent advances have provided a great variety of techniques designed to assess the normal and disordered physiology of the thyroid gland. By and large, these procedures supplement rather than replace one another, and, when employed together, they make possible an integrated analysis of diverse aspects of thyroidal function and the metabolism of iodine. Thus, it is possible to assess the avidity of the thyroid gland for iodine, the quantity of thyroid hormone produced, its rate of release from the gland, the rate of peripheral degradation of hormone, and the concentration and chemical nature of hormonal iodine in the blood.[1,2]

Subacute, giant cell, or de Quervain's thyroiditis is a disease which is puzzling both in its etiology and in its pathologic physiology. In this disease, commonly employed measures of thyroidal function frequently display a seemingly paradoxical divergence from normality. For example, although the thyroidal uptake of radioiodine is characteristically diminished,[3,4] the concentration of circulating hormonal iodine and the basal metabolic rate may be increased, decreased, or normal.[5]

The relative rarity of subacute thyroiditis has prevented physiological studies in large numbers of patients with this disease. It has been generally assumed, however, that the confusing complex of thyroidal function tests could be largely explained by impairment of the formation of new hormone, together with the passage of preformed stores of hormone through disrupted follicular walls into the circulation.[6,7] During the past six years, as various methods have been developed, they have been employed by us to study the metabolism of iodine in patients with subacute thyroiditis. During this period, 10 patients demonstrating the commonly accepted clinical and laboratory diagnostic criteria have been observed. Data obtained in these patients have provided a basis for verification and extension of earlier hypotheses concerning the functional abnormality in patients with this disease. These findings have been previously described in abstract form.[8]

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From the Department of Biophysics, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D. C.; the Thorndike Memorial Laboratory and Second and Fourth (Harvard) Medical Services, Boston City Hospital; the Department of Medicine, Harvard Medical School, Boston, and the Howard Hughes Medical Institute. Formerly Captain (MC), U. S. Army; at present, Investigator, Howard Hughes Medical Institute (Drs. Ingbar and Freinkel).

Materials and Methods

(a) Patient Material.—The group studied was composed of ward patients of either the Walter Reed Army Hospital or the Boston City Hospital. All patients demonstrated the usual historical and physical findings of subacute thyroiditis, namely, pain and tenderness over the thyroid gland, frequently radiating to the jaw or occiput, pain on swallowing, marked asthenia, and, in some instances, chilly sensations and fever. Several patients complained of symptoms of hypermetabolism, including nervousness, increased sweating, and weight loss, either at the time of examination or at some time earlier in the course of the disease. Of 10 patients, all but 2 (Patients 9, 10) had

received antibiotics shortly after the onset of symptoms.

(b) Laboratory Methods.—In Patients 1 to 4, the thyroidal accumulation of radioiodine was measured during the first 4 and at 24 hours after oral administration of 50μc. of carrier-free I^{131}. In the remaining six patients, similar measurements of thyroidal uptake were obtained, except that radioiodine was administered intravenously and that Patients 8, 9, and 10 were given 150μc. of the radioactive isotope.

In all patients, blood was drawn at 24 and 72 hours after the administration of inorganic I^{131}, and the concentration of butanol-extractable I^{131} (BEI^{131}, presumably representing iodinated thyronine moieties) was determined as described elsewhere.[9] Briefly, this consisted of extraction of acidified serum with water-saturated butanol, followed by multiple washings of the butanol extract with alkaline sodium carbonate (Blau's) solution. The final butanol extracts were evaporated under reduced pressure, taken up in a small volume of butanol, and counted in a well type scintillation counter. The radioactivity of the extracts was compared with that of a suitably diluted aliquot of the administered dose. In Patients 8, 9, and 10, measurements were also made of the total radioactivity of the serum and of the protein-precipitable, butanol-nonextractable radioactivity. The latter measurement was made after transfer of the extracted precipitate to a clean counting tube, as it had been shown that a portion of the apparently nonextractable radioactivity was derived from adsorption of radioactive material to glassware.

In 7 of 10 patients, measurements were made of serum precipitable stable iodine (PBI^{127}) in blood drawn at the time the radioiodine studies were performed. In addition, in Patients 9 and 10, the concentration of butanol-extractable I^{127} (BEI^{127}) was also measured.[10] *

Immunologic techniques were employed in an attempt to characterize the butanol-insoluble iodinated moieties in the serum of three patients. Specimens of human thyroid gland were obtained at surgery from patients with either nontoxic multinodular goiter or with treated Graves' disease. From these tissues, purified thyroglobulin was prepared by the method of Derrien, Michel, and Roche.[11] The purified protein was adjusted to a concentration of 1.3 gm. per 100 ml., with use of a modified biuret technique for the measurement of protein concentration.[12] This solution of thyroglobulin was mixed with an equal volume of a suspension of aluminum hydroxide. Two rabbits were then given six injections of the mixture, each containing 6.5 mg. of protein, over a two-week period. After an interval of two weeks, the rabbits were given three similar injections during one week and were exsanguinated seven days later. The rabbit anti-human-thyroglobulin serum thus obtained was found to contain precipitating antibodies against protein of normal human serum, but these constituents could be removed by reacting the rabbit antiserum with an equal volume of a twofold saline dilution of normal human serum. The supernatant remaining after centrifugation and discard of the resulting precipitate was found to react only with human thyroglobulin and to yield a visible precipitin reaction when the thyroglobulin was diluted to a concentration of 1.3 mg. per 100 ml. Precipitin tests for thyroglobulin were performed in serum obtained from Patients 9 and 10 and also from another patient with subacute thyroiditis, not available for radioactive iodine studies. Additionally, these sera were reacted with solutions of purified human thyroglobulin in an effort to demonstrate autoantibodies against this protein.

(c) Therapy.—Eight of the 10 patients were treated by the administration of external radiation to the thyroid area. The radiation dose varied between 500 and 850 r, measured in air.[13] One patient was treated with intramuscularly administered cortisone. Another received thyroid-stimulating hormone (10 units daily for three days). These therapeutic regimens were followed by remission of symptoms. Radioactive iodine studies were repeated within two to four weeks after restoration of an apparently normal state.

Results

Individual data are presented in the Table. Of the 10 patients, 8 were women and 2 were men. Except in the case of Patient 9, who was 76 years old, ages ranged between 34 and 45 years. Duration of symptoms at the time of the initial study ranged between two weeks and four months, averaging eight weeks.

All patients demonstrated a moderate or marked increase in erythrocyte sedimentation rate (Wintrobe method). Sedimentation rates, corrected for hematocrit, ranged between 27 and 38 mm. per hour and averaged 35 mm. per hour.

Distinct increases in basal metabolic rate (BMR) were noted in three patients (Patients 1, 2, and 10). Patient 1 was febrile at the time tests were performed. BMR's averaged +14% and ranged between −7%

* Performed in the laboratory of Dr. E. B. Man, Yale University.

Summary of Data Obtained Before and After Therapy in Patients with Subacute Thyroiditis *

Patient	Age	Sex	Duration, Wk.	ESR, Mm./Hr.	BMR	24-Hr. I^{131} Uptake, % Dose	PBI, μg./100 Cc.	BEI ^{131}, % Dose/L. 24-Hr.	BEI ^{131}, % Dose/L. 72-Hr.	Therapy	Interval Wk.†
1	35	F	8	36	+27	2	2.7	0.071	0.000	X-ray	2
				14	+12	22	4.3	0.042	0.073	700 r	
2	41	F	16	38	+32	2	10.1	0.233	0.119	X-ray	4
				15	+8	16	6.3	0.074	0.104	500 r	
3	45	F	8	36	+4	6	--	0.105	0.006	X-ray	3
				10	+7	22	--	0.057	0.080	700 r	
4	45	F	12	38	+15	2	--	0.043	0.026	X-ray	3
				12	−7	18	--	0.016	0.048	700 r	
5	34	F	8	27	+7	3	--	0.065	0.027	X-ray	3
				9	−2	30	--	0.068	0.085	700 r	
6	43	M	12	35	+14	3	7.3	0.025	0.002	X-ray	3
				7	−20	29	3.8	0.084	0.227	700 r	
7	38	M	2	38	−7	0	4.3	0.020	0.005	TSH	3
				20	+12	11	4.7	0.124	0.043	30 units	
8	38	F	12	34	+15	2	8.9	0.088	0.004	X-ray	4
				18	−13	26	5.7	0.032	0.066	500 r	
9	76	F	3	29	+10	4	16.2 (11.2) ‡	0.030	0.011	X-ray	3
				20	+8	28	4.3	0.024	0.063	850 r	
10	39	F	3	38	+24	8	16.0 (13.7) ‡	0.123	0.042	Cortisone, 200 mg./day ×7	3
				16	+14	38	5.2	0.128	0.242		

* For explanation of symbols, see text. For each patient upper and lower figures in each column represent values obtained before and after treatment, respectively.
† Interval between the end of treatment and post-therapeutic tests.
‡ Concomitantly estimated value of the butanol-extractable I^{127}.

and +32%. Following therapy, mean BMR decreased to +2%. Decreases in BMR were noted in 7 of 10 patients following treatment, but only one value (−20% in Patient 6) was noted to be outside of the normal range.

In the seven patients in whom the measurement was made, values of the PBI^{127} prior to therapy ranged broadly between 2.7μg. and 16.2μg. per 100 ml. Four of seven values exceeded 8μg. per 100 ml., and one of the seven was less than 3.0μg. per 100 ml. Following treatment, normal values for PBI^{127} were found in all patients.

Twenty-four-hour thyroidal uptakes of I^{131} were negligible or markedly diminished in all patients prior to treatment. Values averaged 3%, and no value exceeded 8% of the administered dose. Values in excess of the 24-hour uptake were not seen in any patient during the 4-hour interval following the administration of I^{131}. Following therapy, values for the thyroidal uptake of I^{131} were normal in all cases, except Patient 7. This patient, whose uptake following treatment was only 10%, had noted an amelioration of symptoms coincident with the administration of thyroid-stimulating hormone (TSH).

In all patients prior to therapy, two distinct abnormalities were noted in values for the butanol-extractable I^{131} in serum. Normally, during the period between 24 and 72 hours after the administration of radioiodine the concentration of BEI^{131} in serum increases from a mean of 0.016% to a mean of 0.037% of the dose per liter of serum.[9] In all patients with active subacute thyroiditis, values of BEI^{131} were greater at 24 hours than at 72 hours. Absolute values of the BEI^{131} varied greatly. Despite the marked reduction in the thyroidal accumulation of radioiodine, maximum values for BEI^{131} (24-hour values) were equal to or greater than values seen in normal patients at 72 hours. Twenty-four-hour values averaged 0.080% of the dose per liter. Seventy-two-hour values in 5 of the 10 patients were below the normal range, and for the entire group averaged 0.024% per liter.

Concentrations of BEI^{131} were entirely normal in six of eight patients following treatment by x-radiation. Within the irradiated group, values for the BEI^{131} were slightly in excess of normal in Patient 2 and were in the distinctly hyperthyroid range in Patient 6. Nevertheless, in all patients given x-radiation the normal quantitative relationship between 24- and 72-hour

values were found. This relationship was also normal in Patient 10, who had been treated with cortisone. However, in this patient, absolute values of the BEI^{131} after therapy were in the distinctly hyperthyroid range. Patient 7, whose improvement was concomitant with the administration of TSH, continued to show an inversion of the usual relationship between 24- and 72-hour values of the BEI^{131}, and the 24-hour values were in the distinctly hyperthyroid range.

In Patients 8, 9, and 10, measurements of the concentration of protein-precipitable, butanol-nonextractable I^{131} in serum were made prior to and after therapy. In these patients, at 72 hours after administration of the dose, 33%, 58%, and 44% of the serum-precipitable radioactivity was not extractable with butanol. Corresponding values following treatment were 8%, 7%, and 13%, respectively. In normal serum concomitantly tested, at least 90% of endogenously synthesized, precipitable radioactivity in the serum was butanol-extractable.

In Patients 9 and 10 prior to therapy, aliquots of a single specimen of plasma were concurrently assayed for BEI^{127} and PBI^{127}. These studies revealed in Patient 9, a BEI^{127} of 11.2μg. per 100 ml., with a PBI^{127} of 16.2μg. per 100 ml. In Patient 10, the BEI^{127} was 13.7 and the PBI^{127} 16.0μg. per 100 cc. Measurements of BEI^{127} were not made after therapy. However PBI^{127}'s had diminished to 4.3 μg. and 5.2μg. per 100 ml., respectively.

No precipitin reaction was demonstrable when the sera of Patients 9 and 10 (and of another patient not shown in the Table) were incubated with rabbit anti-human-thyroglobulin serum from which antibodies against normal human serum protein had been removed. Similarly, no antibodies to thyroglobulin could be detected when the sera of these same patients were reacted with solutions of purified human thyroglobulin.

Comment

Perhaps more than any other disease, subacute thyroiditis emphasizes the need for caution in interpreting the results of isolated tests of thyroid function. In this disease, commonly employed laboratory tests often diverge from normal in seemingly paradoxical directions. In the present study, a variety of techniques have been employed to assess the function of the thyroid gland and the metabolism of iodine in patients with subacute thyroiditis. Abnormalities in previously measured functions have been confirmed, and new parameters have been explored in order to elucidate further the pathological physiology of the thyroid gland in this disease.

In confirmation of earlier reports, the 24-hour thyroidal uptake of radioiodine has been found to be markedly decreased during the active inflammatory phase.[3,4] In general, marked reduction or abolition of the 24-hour thyroidal uptake of radioiodine indicates the virtual cessation of accumulation of iodine for the synthesis of new hormone. However, measurement of the radioiodine content of the thyroid at only one point in time gives evidence solely of the quantity of isotope present in the gland at the moment the measurement is made. Under certain circumstances, considerable radioiodine may pass into the gland and be secreted as radioactive hormone by the time 24 hours after the administration of the dose have elapsed. Such rapid cycling of iodine through the gland may be seen in patients with severe thyrotoxicosis and also in occasional patients with markedly hyperactive, though nonthyrotoxic glands, usually following subtotal thyroidectomy.[9,14]

In order to assess the possibility that, by the end of 24 hours, relatively large quantities of radioiodine may have been accumulated and resecreted by the thyroid glands of patients with subacute thyroiditis, measurements were made of the early phase of iodine accumulation (i. e., that occurring within the first four hours after administration of the dose).[15] Here too, it was impossible to distinguish uptake of significant quantities of I^{131} by the abnormal gland.

Since a history of recent iodine ingestion could be obtained in only 1 of the 10 patients studied, it may be concluded that iodide stores in such patients were not abnormally large. Thus, the diminished percentile accumulation of iodine by the thyroid gland was in fact a reflection of a diminution in the synthesis of new hormone.

In order to ascertain whether organic iodinations were completely abolished or merely greatly decreased, studies were made of the radioiodinated moieties appearing in serum after the administration of I^{131}. Among such materials may be iodinated proteins, in which the iodinated moieties form an integral part of the protein structure, and iodinated amino acids which are loosely bound to the surface of the proteins of plasma. Although both groups of compounds are protein-precipitable, the latter group is largely extracted from the protein precipitate by acid butanol, but the former group is not. When butanol extracts are washed with alkaline carbonate solution, iodotyrosines are largely removed, whereas iodinated thyronine derivatives are not. The term butanol-extractable iodine, as employed herein, refers to compounds remaining in the butanol extract after alkaline washing and therefore in most circumstances to iodinated thyronines, including thyroxine and triiodothyronine. Earlier studies had defined the range of values for the BEI^{131} to be found in patients with a variety of thyroidal states. Kinetic analysis of the results led to the conclusion that the concentration of butanol-extractable materials in the serum was less dependent on their rate of synthesis within the thyroid gland than upon their rate of release from the gland, once formed.[9]

Significant quantities of butanol-extractable radioiodinated materials were found in the serum of all patients with active subacute thyroiditis. This indicated that despite the marked reduction in thyroidal uptake of I^{131}, some organic iodinations, presumably thyroidal in locus, were taking place. However, values for the BEI^{131} in patients with subacute thyroiditis were distinctly unusual in two respects. First, values for the concentration of BEI^{131} in the serum declined in all persons during the period between 24 and 72 hours after the administration of the dose. This stands in marked contradistinction to findings in normal or hyperthyroid patients or patients with thyroidal hypofunction. In the latter groups, the 72-hour concentration of BEI is regularly greater than that found at 24 hours. Secondly, absolute values of the BEI^{131} in all patients were well within the normal or hyperfunctioning range, despite the marked reduction in thyroidal accumulation of radioiodine. This complex of findings is best explained by the thyroidal synthesis of small amounts of iodinated materials which are then rapidly released from the gland.

In the face of diminished rates of synthesis of new hormone, increases in the concentration of protein-bound iodine in the circulation, noted by other authors and seen in four of the patients of the present series, seem at first paradoxical. However, the thyroid gland is unique among the endocrines in maintaining a large store of hormone. This can serve as a continued source of hormone for the periphery, despite reduction or complete cessation of hormonal biosynthesis. That this reservoir of preformed hormone is capable of largely preventing a reduction in PBI despite complete suppression of hormonal synthesis is evidenced by the observation that normal patients receiving completely blocking doses of antithyroid drug demonstrate little if any decline in the PBI in serum over a period of 8 to 10 days.[16] It seemed possible, therefore, that maintenance of normal or increased concentrations of PBI in the circulation of patients with subacute thyroiditis was merely a reflection of the release into the circulation of normal or increased quantities of hormone from the glandular pool. If the diminution of new hormone synthesis were to persist for a sufficiently long period, the thyroidal pool of preformed hormone would

eventually become depleted and the concentration of circulating thyroid hormone would progressively decrease. This sequence of events may account for the reduction in PBI to hypothyroid levels, occasionally seen in patients with subacute thyroiditis and noted in the present study in Patient 1. It is of interest that soon after the onset of thyroiditis, this patient passed through a stage highly suggestive of thyrotoxicosis. It seems possible that abrupt release of large quantities of stored hormone had resulted in the previous thyrotoxic manifestations and had led to a rapid depletion of the glandular pool with a resulting decline in the serum concentration of PBI.†

Release from the inflamed gland of abnormally large quantities of preformed hormone and accompanied increase in the concentration of PBI in the circulation might be expected to result uniformly in a state of hypermetabolism or thyrotoxicosis. That this need not always be the case in patients with subacute thyroiditis is demonstrated by Patients 8 and 9, in whom the PBI was markedly increased but in whom the metabolic rate remained normal. Measurement of the concentration of protein-bound iodine, however, provides no evidence concerning the nature of the iodinated material being measured. Eumetabolism could be maintained in the face of increased PBI if the augumentation of PBI were largely due to the presence in the serum of abnormal products of low calorigenic potency.

Several lines of evidence indicated that in subacute thyroiditis this may be the case. First, although these butanol-extractable radioiodinated moieties might be considered on the basis of their solubility characteristics to represent either thyroxine or triiodothyronine, the rapid decline of their concentration in the circulation between 24 and 72 hours suggested that this might not be the case. Normally, circulating thyroxine disappears from the plasma with a half-time of approximately seven days.[17] If the radioiodinated material of butanol extracts were in fact thyroxine, one would expect that at least 70% of the material present at 24 hours would remain within the circulation at 72 hours. In many patients with subacute thyroiditis, however, the concentration of BEI ^{131} at 72 hours was only a small fraction of that found at 24 hours. This suggested that either the peripheral turnover of normal hormone was markedly accelerated or that an abnormal material with a very rapid rate of peripheral turnover was appearing in butanol extracts.

Further evidence for the occurrence of abnormal iodinated products in the serum of patients with subacute thyroiditis was obtained in Patients 8, 9, and 10. In these patients, comparisons were made of the concentrations of protein-precipitable and butanol-extractable I^{131}. In all three patients, approximately half of the precipitable radioactivity was not extractable with butanol. In contrast, in normal subjects, at least 90% of precipitable radioactivity is butanol-extractable.

The occurrence of an abnormal butanol-extractable iodinated moiety in the serum of patients with subacute thyroiditis was confirmed by the demonstration in Patients 9 and 10 of unusually large discrepancy between the concentrations of butanol-extractable and protein-precipitable stable iodine (I^{127}).[10]

The iodine of thyroglobulin is, of course, protein-precipitable but not extractable with butanol. Since thyroglobulin had been demonstrated in the serum of patients with radiation thyroiditis,[18] it seemed possible that the abnormal materials appearing in the serum of patients with subacute thyroiditis might also prove to be thyroglobulin. However, efforts to demonstrate thyroglobulin by immunologic techniques in the serum of three patients with active disease were unsuccessful. The antiserum employed was capable of detecting approximately 1.3 mg. of thyroglobulin per 100 ml. of sera. Assuming an iodine nitrogen ratio in thyro-

† The increased BMR noted in this patient at the time of testing was probably the result of fever (oral temperature, 101 F).

globulin of 4:150[11] and assuming the discrepancy between BEI^{127} and PBI^{127} in the sera of Patients 9 and 10 to consist solely of thyroglobulin, one can calculate an expected thyroglobulin content of approximately 150μg. of nitrogen or approximately 1 mg. of protein per 100 ml. of serum. This was obviously at the lower limit of detectability with the antiserum employed. Similarly, no autoantibodies against human thyroglobulin could be detected in the sera of patients with active disease.

From the foregoing data, gathered in patients with subacute thyroiditis, it is possible to construct a hypothesis concerning the nature of the functional abnormality in this disease. However, the data are best considered in relation to both the normal sequence of hormonal biosynthesis and release and to the glandular pathologic anatomy during subacute thyroiditis.

During normal hormonal biosynthesis, formation of hormonally active iodinated amino acids apparently takes place within the matrix of the thyroglobulin molecule.[19] Intact follicular epithelium serves to contain this large iodinated protein within the follicular lumina. Thus, products of iodination are retained within the follicle until hydrolysis of protein occurs under the influence of thyroidal protease and peptidases.[20,21]

Histologic changes in the gland during subacute thyroiditis would suggest, however, that during this disease, the retentive capacity and storage function of the gland are lost. Destruction of follicular epithelium is accompanied by infiltration of the interstitium and follicular lumina with inflammatory cells and histiocytes. Colloid-like material is seen penetrating the interstitium and being ingested by the multinucleated foreign-body giant cells which give the disease the name, "giant cell thyroiditis."[7,13]

During subacute thyroiditis, formation of new thyroid hormone is markedly diminished, although not completely abolished. Loss of follicular integrity may permit the passage of variable quantities of intact iodinated proteins (both newly formed and preformed) and their partially hydrolyzed products into the thyroidal interstitium. There, in turn, these may be acted upon by the proteases and peptidases of inflammatory leukocytes.[22] There may thus penetrate the circulation varying quantities of a variety of proteins, proteoses, peptides, and amino acids. These would be expected to have widely differing properties with regard to protein-precipitability, butanol-extractability, peripheral turnover, and calorigenic effect. Partial hydrolysis of thyroglobulin might also alter its immunologic reactivity and thereby account for the failure to detect thyroglobulin by immunologic means.

The foregoing sequence of events is of course conjectural but appears to be consistent with the present data. It may be anticipated that considerable difficulty will be encountered in obtaining proof or disproof of this hypothesis. First, the relative rarity of the disease, at least on the Eastern Seaboard makes patient material very scant indeed. Secondly, since the disease is self-limited, administration of more than tracer quantities or radioiodine is not justified. Thus, such potentially powerful tools as chromotography and electrophoresis, both of which could contribute greatly toward the identification of the iodinated materials in the serum, can be employed only with great difficulty, if at all.

The present findings also provide the first data concerning the rapidity and completeness of the return of normal thyroidal function following treatment with x-rays. In addition to a prompt and dramatic improvement in symptoms, x-radiation was followed within a maximum period of four weeks by a return of functional studies to normal or virtually normal values. Although cortisone has proven remarkably effective in controlling the symptoms of subacute thyroiditis,[23,24] comparable demonstration of its ability to restore thyroidal function to normal has not been reported. It seems reasonable that final judgment concerning

optimum therapy in this disease should await such data.

Summary and Conclusions

Studies of thyroid function and the metabolism of iodine have been performed before and after treatment in 10 patients with subacute thyroiditis.

From an analysis of the results, a hypothesis concerning the nature of the functional abnormality in this disease has been formulated.

It is suggested that during subacute thyroiditis, two principal abnormalities in thyroid function occur. The first is a diminution, though not complete cessation of hormonal biosynthesis. The second is a loss of the storage function of the gland, with passage into the circulation of a variety of iodinated materials.

These functional abnormalities have been shown to be promptly restored to normal following x-ray therapy.

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Hemostatic Data in Relatives of Hemophiliacs A and B

Evidence for Modifying the Classical Sex-Linked Recessive Hypothesis

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In 1803, Dr. John C. Otto,[1] of Philadelphia, reported observations on a familial hemorrhagic disorder affecting only males but transmissable via some of the unaffected females. Otto's observations undoubtedly concerned patients whom we now speak of as having hemophilia A (classical hemophilia, antihemophilic factor [AHF] deficiency) or hemophilia B (Christmas disease,[2] plasma thromboplastin component [PTC] deficiency[3]), the genetics of these two disorders being apparently identical. Thus the term "sex-linked recessive" has been applied to both conditions, and by and large the female carrier or heterozygote in such families is asymptomatic and undetectable by most laboratory tests. However, the data of a number of investigators[4-9] who have studied a few such carriers by means of recently developed tests suggest that the carrier state is attended in some cases with both clinical and laboratory manifestations of a mild hemorrhagic disorder. In an attempt to shed further light on this problem a large series of such carriers was studied.

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Materials and Methods

Venous blood for clotting times was taken from the second of two siliconed syringes, the first being delivered into various appropriate anticoagulants.[10] Glass and silicone clotting time, prothrombin consumption, and AHF and PTC assays were performed as previously described.[10] Two-hour prothrombin consumption was performed following Dreskin's[11] modification of Quick's[12] original description, except that blood used as the source of serum was incubated at 37 C for two hours rather than one hour following collection. It is referred to in the text as the "two-hour serum prothrombin time" method. Thromboplastin generation was modified from Biggs and Douglas,[13] with use of equal volumes of 1:5 dilution of $BaSO_4$-adsorbed plasma, 1:10 dilution of serum, and a suspension of platelets[10] whose concentration approximates that in whole blood. Thromboplastin generation, with use of all three components from the carrier, was performed in 13 carriers, but the range of results was wide, as might be expected from the nonspecificity of the test. Therefore, the test was then performed, in the case of hemophilia A carriers, with use of the carrier's $BaSO_4$-adsorbed plasma and normal serum and platelets, and in the case of hemophilia B carriers, with use of the carrier's serum and normal $BaSO_4$-adsorbed plasma and platelets. Thus the method becomes an assay for AHF and PTC, respectively, as long as proaccelerin and proconvertin (Stuart), respectively, are normal in the carriers, and this was found to be the case by appropriate one-stage assays. This method is referred to in the text as "thromboplastin generation assay" for AHF or PTC.

The normal distribution for results in the various tests was calculated from observations in 75 male and 25 female healthy medical students and technicians in Pittsburgh and Chapel Hill, except in the case of the thromboplastin generation and two-hour serum prothrombin time methods, where 25 Pittsburgh males and females were the normal group. No difference based on sex or geographic location was observed. An AHF or PTC level of 45% or less has been considered abnormal, although two "normal" subjects were found, each

with a level of 45% for AHF or PTC, respectively. In an additional group, of approximately 100 medical students, no levels were found below 60%.

Hemophilia A and B carriers comprised, respectively, 63 females from 43 families and 14 females from 11 families, who were "obligatory carriers," i. e., either (*a*) mothers of more than one hemophiliac male, (*b*) mothers of more than one carrier, (*c*) mothers of at least one hemophiliac male and one carrier, (*d*) daughters of a hemophiliac male, or (*e*) mothers of a single hemophiliac male or of a single carrier if one of the mother's siblings is also a hemophiliac male or carrier. Each carrier was related to at least one male subject studied by us and meeting the criteria of hemophilia A or B: (*a*) hemorrhagic symptoms since infancy or childhood, (*b*) specific AHF or PTC assay below 20% of normal, and (*c*) all other coagulation factors, as well as bleeding time (Duke or Ivy) and Rumpel-Leede test,

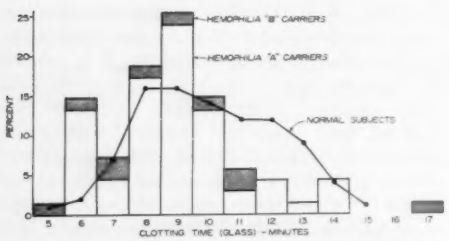

Fig. 1.—Distribution of glass clotting times in normal subjects and in carriers of hemophilia A and B.

normal. An average of the clinical and the laboratory severity of each hemophiliac was calculated (there is usually close correlation between the two) and a figure of 1+ to 4+ was assigned, based upon the following criteria: 1+ indicates hemorrhagic symptoms only following trauma or surgery, 10% to 20% AHF or PTC; 2+, one major hemorrhagic episode yearly without trauma or surgery, 1% to 10% AHF or PTC; 3+, two to four such episodes yearly, 0.2% to 1% AHF or PTC; 4+, five or more such episodes yearly. <0.2% AHF or PTC.

Results

Figure 1 shows the distribution of glass clotting times of hemophilia A carriers, hemophilia B carriers, and normal subjects. All carriers fell within the normal range except for one hemophila B carrier, who was just above the upper limit of normal. Figure 2 shows the silicone clotting time

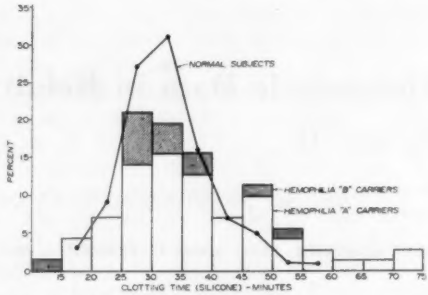

Fig. 2.—Distribution of silicone clotting times in normal subjects and in carriers of hemophilia A and B.

data. Results in four hemophila A carriers were beyond the normal range.

In Figure 3 are shown the results of the one-hour prothrombin consumption test. Here a slight "shift to the left" can be noted. Four hemophilia A and one B carrier were subnormal, resulting in a total of 8.8% below the normal range. Figure 4 shows data with use of the two-hour serum prothrombin time method. Again a slight "shift to the left" is demonstrated; 10.5% of the whole group showed results lower than the lower limit of normal.

In Table 1 the results of AHF or PTC assays in 216 female and 74 male relatives of hemophiliacs are summarized. The mean AHF or PTC level in obligatory carriers and mothers of one hemophiliac child are significantly lower than those for males and

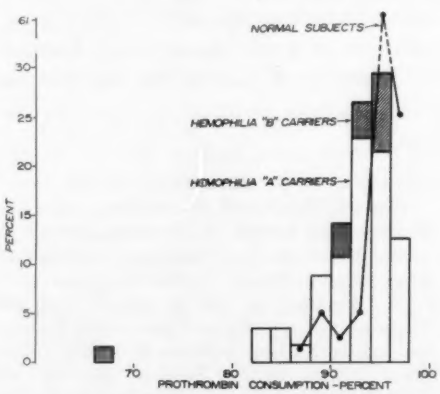

Fig. 3.—Distribution of prothrombin consumption in normal subjects and in carriers of hemophilia A and B.

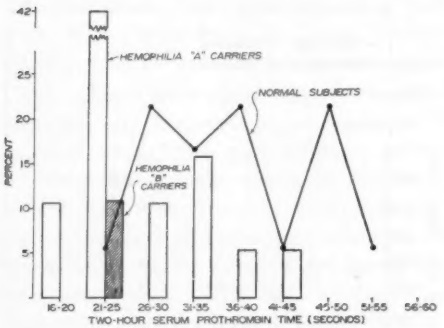

Fig. 4.—Distribution of two-hour serum prothrombin times in normal subjects and in carriers of hemophilia A and B.

normals. In the fourth column are listed the number with levels of 45% or less. Again this differs markedly from the consistently normal levels seen in male relatives. Carriers of PTC deficiency appear to be more frequently "abnormal" by laboratory test than those of AHF deficiency. PTC assays performed on hemophilia A carriers all fell within the normal range, as did AHF assays done on hemophilia B carriers.

In Table 2 two hemophilia A carriers, one who had been found to have a normal AHF level (100%) and the other, a low level (25%), were tested with use of various sources of plasma as the substrates, i. e., their own hemophiliac son's, each other's son's, and another severe hemophiliac's, unrelated to either carrier whose plasma has served as the standard substrate throughout. Carrier I's AHF level, which was normal when tested against her own son (Patient 1), also assayed normally when tested against the other two hemophiliac plasmas. Likewise, Carrier II's AHF level, subnormal when her son's plasma was used as substrate, remained low when the two other hemophiliac plasmas were used.

Figure 5 demonstrates the fact that on repeated testings during a period of over a year, carriers of hemophilia A or B who assayed low in AHF or PTC, respectively (M. L., R. L.), remained low on each occasion; likewise, those carriers who had a normal assay once (S. A., G. M.) had it every time they were subsequently tested. Forty per cent of the total group of carriers were tested more than once, and sixteen per cent, more than twice, with little variability in results from one examination to the next.

The "thromboplastin generation assay" for AHF was performed on 29 carriers of hemophilia A. Five of these 29 (17.2%)

TABLE 1.—*AHF or PTC Levels in Plasma from Relatives of Hemophiliacs A and B*

Classification	No. Studied	AHF or PTC, %		AHF or PTC (45% or Less)	
		Mean	Range	No.	%
Obligatory carriers	77			12	15.6
AHF	63	76	10-120	8	12.7
PTC	14	62	10-100	4	28.6
Possible carriers	139			15	10.8
Mothers (1 hemophiliac)	29			9	31.0
AHF	24	67	12-100	6	25.0
PTC	5	48	20-80	3	60.0
Sisters, cousins, etc.	110			6	5.5
AHF	90	94	10-150	5	5.6
PTC	20	85	40-125	1	5.0
Males (Fathers, brothers, sons, cousins)	74	100		0	0
AHF	60	100	50-200	0	0
PTC	14	101	80-125	0	0
Normal subjects	80	100		2	2.5
AHF	80	100	45-220	1	1.3
PTC	80	100	45-150	1	1.3

TABLE 2.—*AHF Assay* *

Subject Tested	Substrate—Hemophilia "A" Plasma from		
	Patient 1	Patient 2	Patient 3
Normal	100	100	100
Hemophilia "A" Carrier I	100	80	125
Hemophilia "A" Carrier II	25	30	30
Patient 1 †	0	0	0
Patient 2 ‡	0	0	0

* Values given as per cent.
† Son of Carrier I.
‡ Son of Carrier II.

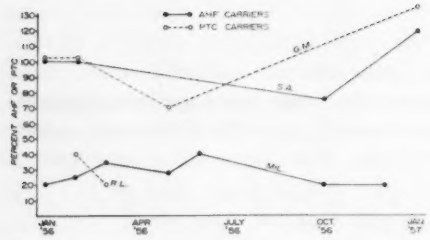

Fig. 5.—Assays of AHF or PTC in carriers tested over a 12-month period.

gave subnormal results. By dilutions of normal $BaSO_4$-adsorbed plasma the range of results was found to be 25% to 115%, the normal range being 65% to 135%. Two carriers of hemophilia B were studied by the "thromboplastin generation assay" method for PTC: the results were within the normal range.

Figure 6 shows the correlation between results obtained with the specific assay for AHF (or PTC) and with the "thromboplastin generation assay" for AHF (or PTC) in all carriers in whom both tests were performed. The correlation coefficient (r) between these two tests is 0.631.

In Figure 7 are shown the results of the thromboplastin generation assay test for AHF in hemophilia A Carriers I and II and their hemophiliac sons, Patients 1 and 2. These are the same four persons who were represented on Table 2. The curve for the normal subject levels off at 10 to 11 seconds. Carrier I is seen to have completely normal thromboplastin generation. However, Carrier II is grossly defective, never coming

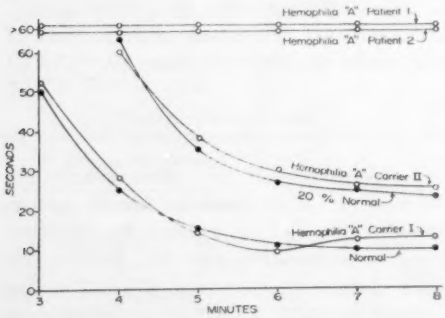

Fig. 7.—Thromboplastin generation AHF assays in Carriers I and II (normal serum and platelets).

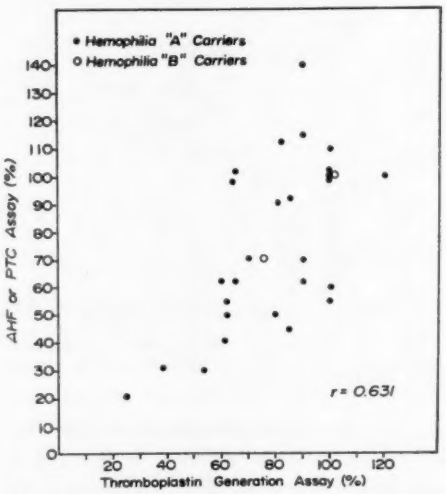

Fig. 6.—Correlation between AHF or PTC as assayed by specific method and thromboplastin generation method.

below about 22 seconds. Dilution of normal plasma with hemophilic plasma enables the calculation of 20% as her AHF level. Despite this difference of these two carriers, their hemophilic sons are identical in being capable of no appreciable thromboplastin generation during the eight-minute incubation period, as is indicated by the two straight lines at the top of the figure.

In Figure 8, the thromboplastin generation curve (not the assay) of one of the "abnormal" carriers of hemophilia A is shown.

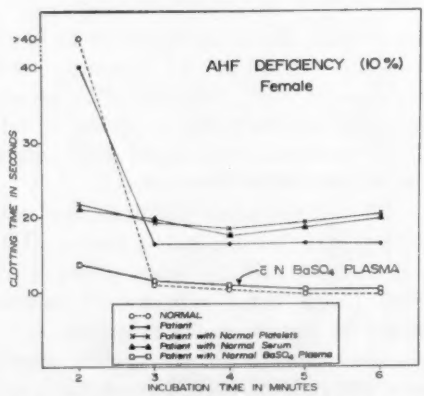

Fig. 8.—Thromboplastin generation test in hemophilia A carrier, who assayed 10% AHF by specific test.

The pattern is identical to that of a male with mild hemophilia A; only normal $BaSO_4$ plasma corrects the curve to normal.

All carriers were asked whether they had ever had any hemorrhagic phenomena. Of the total group, 28% gave a history of one or more hemorrhagic symptom, which appeared to be in excess of normal. However, among those carriers who were abnormal on the basis of one or more of the laboratory tests performed, 52% had a significant hemorrhagic history, as compared with 20% of the carriers whose tests were all normal. Significant hemorrhagic symptoms were observed in both the hemophilia A and the hemophilia B carrier groups. Among these 52%, the commonest symptoms were excessive bleeding following operation, tooth extraction, and delivery. Many had required transfusions to replace blood lost, and, in two, intravenous plasma therapy was given pre- and postoperatively, with apparent excellent results in preventing hemorrhage and raising AHF levels almost to normal range. Other symptoms recorded by this group, in decreasing order of frequency, were menorrhagia, ecchymoses following minimal or no trauma, bleeding from gums, and epistaxis. Hematuria, gastrointestinal bleeding, and hemarthroses were not observed. Hemorrhagic symptoms among carriers whose laboratory tests were normal were in general less severe than those of the above group.

Those carriers who have a laboratory abnormality do not predominantly come from families afflicted with mild hemophilia or from families with severe hemophilia; on the contrary, the four gradations of laboratory and clinical severity (Materials and Methods) are about equally represented here. In addition, when several obligatory carriers in a family were studied and one was found abnormal, the other(s) were not necessarily abnormal.

Comment

These results indicate that clotting time in glass is essentially valueless for the detection of the minor deficiencies present in some of the carriers. This fact fits well with our knowledge of the degree of sensitivity of the test, namely, that 3% to 4% AHF or PTC is enough to result in a normal glass clotting time, other clotting factors all being normal. With use of the silicone clotting time, 5.2% of the carrier group were abnormal, while the one- and two-hour prothrombin consumption methods gave 8.8% and 10.5% abnormal results, respectively.

Of greater interest were results using the specific AHF and PTC assays and the "thromboplastin generation assays." If the mothers of one hemophiliac child are included with the obligatory carriers, 14 of the 87 hemophilia A carriers and 7 of 19 hemophilia B carriers showed subnormal AHF or PTC assays, respectively, resulting in an over-all figure of 19.8% abnormal. At present the series is too short to tell whether or not the higher proportion of abnormal carriers in hemophilia B than A is significant. The "thromboplastin generation assay" methods for AHF and PTC yielded over-all subnormal results in the same percentage range (16.1%).

The fact (Table 2) that the laboratory "normalness" or "abnormalness" of a carrier is independent of which of a number of plasmas is used, including her own hemophiliac son's, as substrate for the AHF assay is highly suggestive that there is a real difference between "normal" and "abnormal" carriers and that this difference lies in a partial deficiency of AHF in the "abnormal" hemophilia A carriers. Repeatedly similar results on subsequent examinations of both hemophilia A and B carriers over a year's period (Fig. 5) are further evidence of the difference between these two groups and that this is likely to be a congenital deficiency.

The significance of subnormal AHF or PTC assays is emphasized by the results obtained in some 2000 assays on about 1000 persons with and without hemorrhagic disorders. Levels of AHF of 45% or below were found only among the following: 142 patients with hemophilia A; 8 obligatory

and 11 nonobligatory carriers of hemophilia A; 1 nonhemophilic with acquired anti-AHF; 15 patients with "pseudohemophilia A," a familial nonsex-linked disorder characterized by depression of AHF in the range of 10% to 45%, prolonged bleeding time, and often increased vascular fragility, and 1 "normal" female. (Tests for anti-AHF,[10] bleeding time, and vascular fragility were performed in most of the hemophilia A carriers and were normal.) Levels of PTC of 45% or below were found only in the following: 40 patients with hemophilia B, 4 obligatory and 4 nonobligatory carriers of hemophilia B, 30 normal newborn infants (cord blood), 19 patients with severe hepatocellular damage, 10 patients with vitamin K deficiency (including 5 suffering from overdoses of coumarins or other vitamin K antagonists), and 1 "normal" subject.

Though the conclusions of this paper are made only on the basis of the "obligatory carrier" data, it seems likely that most of the mothers of a single hemophiliac or carrier, there being no other history of hemophilia in the family, are genetically identical with the obligatory group. That is to say, most such mothers truly have a positive family history of hemophilia, which has been either forgotten or denied. It seems likely that mutation of the hemophilia A or B genes is so rare an event as to account for only a small percentage of the hemophiliacs occurring in families where no previous history of hemophilia is recorded. No evidence of excessive exposure of these mothers of one bleeder, either in utero or in later life, to noxious influences (x-ray, diseases of their mothers during early pregnancy) could be obtained, although, admittedly, this is a difficult subject upon which to obtain factual evidence. The observation that among the 29 mothers of only one hemophiliac 31% were abnormal, suggests that these mothers were indeed carriers and that, if a mutation of the normal gene (H) to the hemophiliac gene (h) did occur, it was in the mother and not the hemophiliac child.

Of the six other female relatives of hemophiliacs who were abnormal by one or more of the laboratory tests employed, only one has had any children (two boys and one girl). It is tempting to predict, from these tests, that the others (young girls) who on the basis of family history are not necessarily carriers are in fact truly carriers and that the incidence of hemophiliacs or carriers among their offspring will eventually bear this out.

The symptomatology of the carriers offers further support of the thesis that there are two groups of carriers ("normal and abnormal"), for there is a significant differential (20% versus 52%) in frequency of hemorrhagic symptoms between those carriers whose laboratory tests are all normal and those in whom one or more of the tests was abnormal. So the presence of hemorrhagic symptoms in a carrier considerably increases the probability of finding a laboratory abnormality as the presumed basis for her symptoms.

Of the 77 carriers studied, 15 were daughters of hemophiliacs. Excluding from the calculations the one of these 15 who is a hemophilia B carrier, of the 14 remaining hemophilia A carriers, 7 were abnormal and 7 normal from the standpoint of the laboratory tests performed. This 50% division is what would be expected if there were three possible alleles at the hemophilia A locus on the X-chromosome, as has been postulated by Haldane. Graham[14] has reported results similar to the above among daughters of of male subjects with mild hemophilia A, whereas daughters of patients with severe hemophilia A were all normal. Thus he is unable to reconcile such data with a multiple-allele hypothesis. Likewise, the one detectable carrier among 19 obligatory and 8 nonobligatory hemophilia A carriers studied by Margolius and Ratnoff[15] recently was the daughter of a mild bleeder. However, our 14 carriers alluded to above are not exclusively from families of mild hemophilia; on the contrary, 11 of the 14 are from families who by clinical and laboratory

standards have moderate to very severe hemophilia A.

The fact that only a small percentage of all carriers is detectable by present-day laboratory methods can mean either one of two things: (1) that these methods are not sensitive enough yet to detect all the other carriers but some day will be so with advanced techniques or (2) that more than this small per cent of the total body of carriers will never be detectable because the "normal" carriers are truly different from the "abnormal" ones from the standpoint of the blood. It certainly seems indisputable that some carriers are repeatedly easily detectable and others repeatedly defy detection, thus suggesting an inherent difference between these two groups. It may be for this very reason, in addition to the limitations of some of the test systems used, that so many conflicting reports,[2,4-9,15-39] with use of various techniques, have been made concerning the detectability of hemophilia A and B carriers.

The finding of mild clinical manifestations among some carriers of hemophilia A and B is not unique among "sex-linked recessive" disorders: color blindness, choroideremia, Leber's disease, and nystagmus are among those conditions cited by Sorsby,[40] in whom varying percentages of the females in these pedigrees are affected.

It seems conceivable on the basis of these data that hemophilia A and B are not completely sex-linked recessive disorders; that is to say, that in a female carrier with the gene combination hH (h indicates hemophiliac; H, normal), h may be (1) recessive, thus allowing H to dominate, resulting in a normal AHF (or PTC) level, or (2) h may not be completely recessive and thus may modify the AHF or PTC level, resulting in a subnormal value. On the other hand, the male, having only the gene h or the gene H, is either hemophilic or normal. Evidence for this is that nonbleeder males in hemophiliac families have AHF or PTC levels within the normal range.

Summary

Sixty-three obligatory carriers of hemophilia A and 14 of hemophilia B were studied by means of the available tests of blood coagulation. Of the 63 obligatory hemophilia A carriers, 8 (12.7%) were abnormal on the basis of two or more of the tests used or on two or more occasions. By the same criteria, of the 14 obligatory carriers of hemophilia B studied, 4 (28.6%) were abnormal. The coagulation defect is constant at repeated testings over a year's time. Fifty-two per cent of the obligatory carriers whose laboratory tests were abnormal have had significant hemorrhagic symptoms; 20% of those who were normal also had hemorrhagic symptoms, usually milder in nature.

Genetic implications of these findings are discussed. In some cases the genes h^a for hemophilia A and h^b for hemophilia B appear to be only partially recessive.

Dr. C. C. Li, of the Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, supplied advice regarding genetic considerations. Many other physicians cooperated in this work.

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Studies on the Destruction of Red Blood Cells

XIII. Observations on the Role of pH in the Pathogenesis and Treatment of Painful Crisis in Sickle-Cell Disease

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The chief clinical features of sickle-cell anemia are chronic hemolytic anemia and episodic painful crises; the underlying defect is the presence of S hemoglobin * in the red blood cells from patients with this disease. The deoxygenation of S hemoglobin leads to the formation of irregular elongated molecular masses—tactoids—which give to the red cells the distorted shape from which the disease derives its name.[1,5] The manner in which the sickling phenomenon accounts for the physiological and pathological disturbances that characterize sickle-cell anemia has been a major interest of Castle and his associates.[6,7]

The chronic hemolytic anemia has been explained on the basis of the increased mechanical fragility that accompanies the sickled state,[6,8-10] with diminished life span of the erythrocytes in vivo.[11] The painful crises have been related to the increased viscosity of the blood that is proportional to the degree of sickling.[6,7] Factors which impede the flow of blood increase the likelihood of sickling in vivo and thus may cause increased viscosity of the blood, thereby further decreasing blood flow so that local hypoxia becomes more marked, causing the degree of sickling to increase. Thus is instituted the "vicious cycle" of erythrostasis, increased sickling, increased viscosity, etc. leading to localized ischemia and ultimately to thrombosis with infarction.[6,12]

Red cells containing high concentrations of S hemoglobin may begin to assume the sickled form at oxygen tensions that are commonly found in body tissues,[2,7,10,13] and relatively small decreases in oxygen tension thereafter may increase markedly the degree of sickling in such cells. At the usual hematocrit values that occur in patients with sickle-cell anemia the increase in viscosity that follows the assumption of the sickled form is not great. However, as the hematocrit increases, large increases in viscosity occur when the red cells assume the sickled form; increased hematocrit and increased degree of sickling appear to be necessary for the initiation of the vicious cycle.

Thus, hypoxia or other factors leading to increased sickling may, in combination with vascular stasis, provide a basis for precipitating a crisis. This is contrasted with the asymptomatic sickle-cell trait in which the concentration of S hemoglobin is such that the red cells do not begin to sickle until relatively low levels of oxygen tension have been achieved.[2,7,10,13]

A mechanism by which sickling may be augmented in vivo was suggested by ob-

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* Many early observations suggested that it was the hemoglobin that was abnormal in sickle-cell anemia.[1-3] Pauling has on several public occasions acknowledged the stimulus to his studies of abnormal hemoglobins that came from Castle's suggestion to him that the hemoglobin probably was abnormal in sickle-cell anemia and that newer physical methods of study might detect the abnormality (see also Reference 4).

servations in vitro that lowering the pH of bloods containing S hemoglobin increased the degree of sickling of red cells maintained at constant oxygen tensions.[13] Thus, few if any red cells from patients with sickle-cell anemia were in the sickled form at pH 7.5 and pO_2 of 60 mm. of mercury, whereas more than 90% of the cells were in the sickled form when the same blood at the same oxygen tension was at pH 7.0. These and similar observations suggested that physiologic changes in pH might influence sickling in vivo. The effects of acidosis and alkalosis in patients with sickle-cell disease were therefore studied. The observations to be presented show that induced acidosis may precipitate painful crises in patients with sickle-cell disease and that the administration of alkali may be of benefit in the treatment of crises.

Clinical Material

All of the patients studied were Negroes with characteristic sickle-cell anemia (hemoglobin phenotype: S hemoglobin) with one exception. The red cells of one patient (Patient 2), contained 73% S, 21% A, and 6% F hemoglobin, a pattern that has been called sickle-cell-thalassemia disease.[14] However, since the mother was hematologically normal and the father was not available for study, the genetic background of the hematologic findings could not be confirmed, and the patient is designated as having S-A-F disease.[13] Pertinent clinical and hematologic data are given in Table 1.

Methods

The hematologic methods have been described elsewhere, as has the technique of measurement of the relative viscosity of whole blood.[7,13] The standard conditions for measurement of viscosity at different oxygen tensions were as follows: hematocrit adjusted to 35%, temperature maintained at 37 C, and pCO_2 of 76 mm. of mercury. The increase in relative viscosity of blood at reduced oxygen tensions over that of fully oxygenated blood was determined. It is expressed as the percentage of the maximal increase in viscosity that occurred after complete deoxygenation of the blood. This percentage increase in viscosity is plotted against oxygen tension.

In order to depict serial changes in the susceptibility to sickling of red cells, the percentage increase in the viscosity of blood at an oxygen tension of 60 mm. of mercury is plotted against time. At this oxygen tension, under the standard conditions in vitro, the viscosity changes are greatest with changes in pH [13] in sickle-cell anemia blood.

Blood pH was measured potentiometrically at 37 C in a Cambridge pH meter. The blood was drawn under oil without stasis from an antecubital vein into a syringe containing 1 mg. of heparin for 20 ml. of blood. Blood was also drawn under oil and permitted to clot for determination of the CO_2 content of the serum.[15] Potassium and sodium concentrations in serum and heparinized blood were determined by flame photometery, and chlorides, by the method of Schales and Schales.[16]

Results

Effect of Single Administration of Ammonium Chloride to Asymptomatic Patient with Sickle-Cell Anemia.—Patient 1, who was asymptomatic at the time, received intravenously 0.85 mEq. of ammonium chloride per kilogram of body weight. A total of 42.5 mEq. was administered during a two-hour period. The serum CO_2 content was 26.7 mEq. per liter before the infusion began and was 23.4 mEq. per liter immediately after the infusion had been given. There were no significant changes in the viscosity curves of bloods drawn immediately before and immediately after the infusion. The patient remained asymptomatic throughout the procedure.

Effect of Metabolic Acidosis in Asymptomatic Patient with Sickle-Cell Anemia.—Acidosis was induced on two separate occasions in Patient 1 by the prolonged administration of ammonium chloride and

TABLE 1.—*Hematologic Findings in Patients with Sickle-Cell Disease*

Patient	Age	Sex	RBC/ Cu. Mm.	HB., Gm./100 Cc.	HCT., %	MCV, Cu. μ	MCHC, Gm./100 Cc.	Retic., %	Icterus Index
1	19	F	3,570,000	8.2	26.6	75	31	5.5	15
2	10	F	2,920,000	6.2	20.1	69	31	5.2	5
3	24	F	2,710,000	6.7	22	81	30	15.8	10
4	35	F	3,180,000	8.5	27	85	31	18.6	35
5	32	M	5,450,000	10.6	35	64	30	3.2	12.5

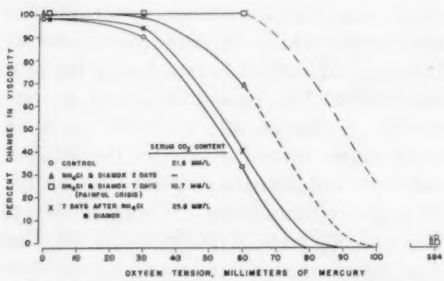

Fig. 1.—Viscosity changes in the blood during metabolic acidosis induced by ammonium chloride and acetazolamide in a patient with sickle-cell anemia.

acetazolamide (Diamox). Before treatment was begun the value for the pH of her venous blood was 7.48 on each of three separate occasions and the serum CO_2 content was 21.6 mM. per liter. The patient was then given by mouth three gm. (1.13 mEq. per kilogram) of ammonium chloride in gelatin capsules every 6 hours and 250 mg. of acetazolamide every 12 hours. The pH of the blood and the CO_2 content of the serum decreased progressively as this treatment continued. On the seventh day of acidification the patient developed severe pains in her back and extremities and stated that the pains were similar to those of a spontaneous crisis. At this time the pH of her venous blood was 7.20 and the CO_2 content of the serum was 10.7 mM. per liter. On the following day, since the pains were not diminished in severity, the ammonium chloride and acetazolamide were discontinued. The symptoms subsided during the subsequent 48 hours and the pH of the blood and the CO_2 content of the serum returned to pretreatment values. During the period of metabolic acidosis the red cells of the patient became more susceptible to the effect of deoxygenation, as indicated by a shift to the right of the viscosity curve (Figure 1).

Seventeen days later, while the patient was asymptomatic, ammonium chloride and acetazolamide were again given in the same dosages. However, the ammonium chloride was given in enteric-coated tablets this time. The pH of the blood fell to 7.30 but did not decrease further during the succeeding seven days. Enteric-coated tablets were recovered in the patient's stool. The ammonium chloride was therefore given in gelatin capsules, whereupon the pH of the blood decreased further and once again the patient experienced severe pains in the extremities and back. The acidifying regimen was discontinued, and the pains subsided gradually during the next 48 hours.

In the second induced crisis, as in the first, the susceptibility to deoxygenation of the patient's red cells increased during the period of acidosis. The relationship of the changes in the viscosity curves to the periods of acidosis is shown in Figure 2, in which is plotted the viscosity ratio that indicates the relative number of red cells that have assumed the sickled form at pO_2 of 60 mm.

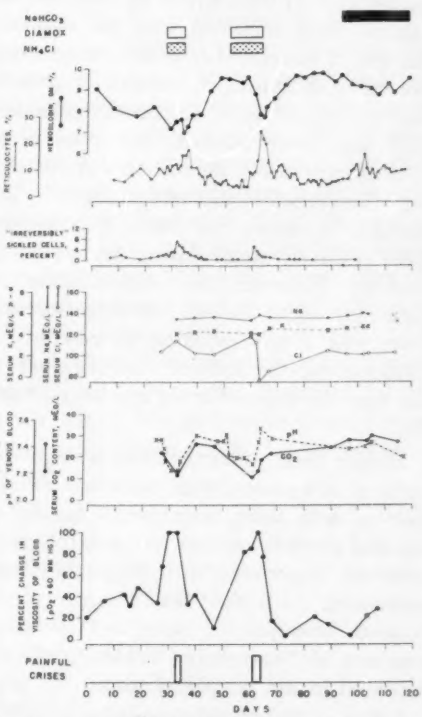

Fig. 2.—Clinical, biochemical, and hematologic effects of metabolic acidosis induced in a patient with sickle-cell anemia. The per cent change in the viscosity of blood at an oxygen tension of 60 mm. of mercury is considered to be an index of the susceptibility to sickling of the red cells (see text).

TABLE 2.—*Chemical Changes in the Blood of a Patient with Sickle-Cell Anemia in Relation to Induced Painful Crisis*

Medication	Clinical Condition: Asymptomatic None	Painful Crisis NH_4Cl and Acetazolamide for 6 days p. o.	Asymptomatic $NaHCO_3$ for 12 days p. o.
pH venous blood	7.42	7.22	7.44
Serum			
CO_2 content, mM./L.	24.6	10.7	28.4
Cl, mEq./L.	101.8	114.8	102.3
Na, mEq./L.	137.0	134.5	138.5
K, mEq./L.	4.6	4.0	4.3
Plasma			
Na, mEq./L.	138.0	132.5	143.7
K, mEq./L.	4.3	3.5	5.0
Whole blood			
Na, mEq./L.	99.5	107.0	102.5
K, mEq./L.	33.1	23.2	28.8
Hematocrit, %	29.3	23.6	29.3
RBC			
Na, mEq./L.	6.8	25.4	3.4
K, mEq./L.	102.5	86.8	86.4
Na + K, mEq./L.	109.3	112.2	89.8

of mercury. Acidosis produced a striking increase in the susceptibility to sickling of the red cells at this oxygen tension, and the painful crisis coincided with the maximal changes in susceptibility to this oxygen tension in vitro. During the periods of acidosis the hemoglobin levels decreased, reticulocytosis was more marked, and there were distinct increases in the numbers of circulating "irreversibly sickled" forms.[17] The changes in serum sodium and potassium were relatively small during the period of acidosis. However, the concentration of potassium within the red cells decreased, and there was a corresponding increase in the intraerythrocytic sodium concentration at the time the induced crisis appeared (Table 2).

Briefly, then, when metabolic acidosis was induced in a patient with sickle-cell anemia, her red cells were more readily sickled at reduced oxygen tensions and painful crises occurred. Associated with the acidosis and consequent crisis there was evidence of increased hemolysis in vivo and increased numbers of "irreversibly sickled" red cells appeared in the peripheral blood.

Effect of Single Administration of Alkali to Patient with Sickle-Cell Anemia.—While Patient 1 was asymptomatic she was given 1.26 mEq. per kilogram of sodium bicarbonate (total dose, 63 mEq.) by mouth in a single dose, and simultaneously an infusion was begun which delivered intravenously 119 mEq. of sodium lactate during the next two hours. The patient remained asymptomatic. However, the viscosity curve of blood drawn immediately after the infusion had been completed was shifted to the left (Fig. 3), indicating less tendency of the red cells to sickle under hypoxic conditions. Two days later, when the viscosity curves were again determined, the patient's blood had returned to its pretreatment pattern. Thus, acute alkalosis caused the blood of this patient to be less susceptible than usual to the effects of deoxygenation.

Effect of Administration of Alkali to Patients Undergoing Spontaneous Painful Crisis.—The possibility that the administration of alkali might provide symptomatic relief during a painful crisis by decreasing the susceptibility of the red cells to sickling was suggested by the preceding observations. This possibility was reinforced by observations in this laboratory indicating that the red cells of some patients undergoing spontaneous painful crises were more likely to sickle at given oxygen tensions than was the case when the patients were asymptomatic (Fig. 4).

Five patients who were undergoing spontaneous painful crises were treated with alkali; one of these patients was treated on two separate occasions, so that six spontaneous crises have been treated. Viscosity studies of the blood were performed in four of these crises before and after the adminis-

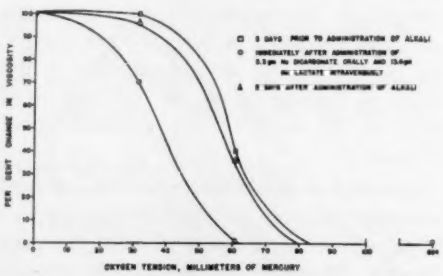

Fig. 3.—Viscosity changes in the blood following the administration of alkali to a patient with sickle-cell anemia.

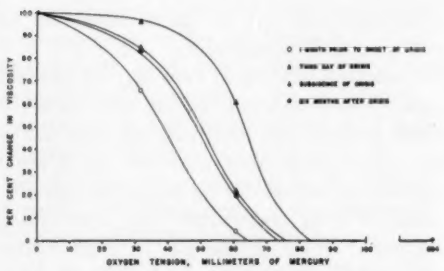

Fig. 4.—Viscosity changes in the blood in relation to spontaneous painful crisis in a patient with sickle cell anemia.

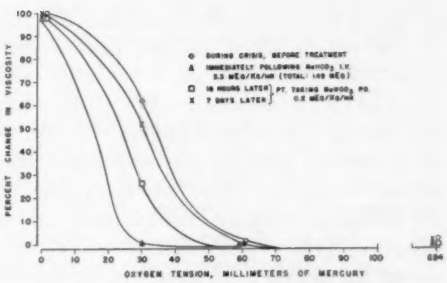

Fig. 5.—Viscosity changes in the blood following the administration of alkali to a patient with S-A-F disease undergoing spontaneous painful crisis.

tration of alkali. In each instance, there was a shift of the viscosity curve in the direction of decreased sickling immediately after the alkali had been given and there was prompt subsidence of the pain. In two crises viscosity curves could not be studied; neither of these responded to treatment with alkali.

Patient 2 was admitted to the Boston City Hospital on the day of onset of a typical painful crisis. She was given 1500 ml. of isotonic saline intravenously over a period of 90 minutes, with no symptomatic response. The saline solution was replaced by a solution containing 134 mEq. of sodium bicarbonate in 1500 ml. of 5% dextrose in water, which was given during the next 90 minutes (3.3 mEq. of sodium bicarbonate per kilogram per hour). Within 60 minutes after the alkaline infusion had begun the patient stopped crying, and after 90 minutes she felt well and asked for food. The viscosity curve of blood drawn before the infusion of saline is compared with that obtained after the infusion of sodium bicarbonate in Figure 5. After the alkaline infusion, the viscosity curves were shifted in the direction indicating decreased susceptibility of the red cells to the effects of hypoxia.

The patient was given by mouth 0.2 mEq. of sodium bicarbonate per kilogram of body weight every four hours for the next seven days after she became asymptomatic. Despite this the viscosity curve returned to the pretreatment values although the painful crisis did not recur.

Two months later, the patient again was admitted with a spontaneous painful crisis of one day's duration. Sodium bicarbonate in the dose of 3.3 mEq. per kilogram per hour was given again for 90 minutes, and again there was prompt subsidence of symptoms coincident with a shift in the viscosity curve in the same direction as before.

A second patient (Patient 1) entered the hospital two days after the onset of a severe painful crisis and received the same dose of sodium bicarbonate over the same period of time as did the preceding patient. The clinical response and the changes in the viscosity curves were similar to those observed in Patient 2 (Fig. 6); the pains were almost completely gone within two hours after the onset of treatment with sodium bicarbonate.

A third patient (Patient 3) also was given sodium bicarbonate for treatment of a spontaneous crisis that she described as the worst that she had suffered in years. Sodium bicarbonate was given in the same dose as

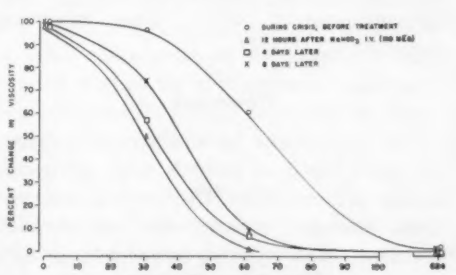

Fig. 6.—Viscosity changes in the blood following the administration of alkali to a patient with sickle-cell anemia undergoing spontaneous painful crisis.

to the previously described patients. The pH of the venous blood of this patient rose from 7.34 before sodium bicarbonate was given to 7.52 immediately after the infusion had ended. There was prompt subsidence of symptoms, and the viscosity curve was shifted to the left after administration of the sodium bicarbonate. For example, before treatment 31% of the patient's red cells assumed the sickled form at pO_2 of 60 mm. of mercury, whereas only 5% of the cells were in the sickled form at the same oxygen tension after treatment.

Two other patients (Patients 4 and 5) each suffered spontaneous crises of one day's duration and were treated with sodium bicarbonate intravenously as above with no symptomatic improvement. Viscosity curves and blood pH data could not be obtained in either of these patients, and it is not known whether a sufficient degree of alkalosis was achieved to alter the viscosity curves of the blood.

Effects of Prolonged Oral Administration of Sodium Bicarbonate to Patients with Sickle-Cell Disease.—Patients 1 and 2 received 6 mEq. per kilogram per day of sodium bicarbonate in divided doses for one month. These were the maximum tolerated doses. Frequent tests showed the urine to be consistently alkaline during the treatment period. No significant changes in the pH of the venous blood, the hemoglobin levels, or the viscosity curves were observed (Fig. 3). There were no significant changes in the concentrations of sodium or of potassium in the plasma, although the intraerythrocytic concentrations of both of these ions were decreased (Table 2).

Comment

The mechanisms by which painful crises are precipitated in patients with sickle-cell disease are not clear. The present observations, however, support the concept that variations in the sickling of red cells in vivo play a key role in the production of the crisis and indicate once again that viscosity studies in vitro may detect physiologically meaningful changes in the susceptibility of red cells to deoxygenation.

Increased sickling in vivo may be brought about in several ways: simple hypoxia is the most obvious, and even patients with sickle-cell trait have been observed to develop splenic infarction during travel at high altitudes.[18] It is noteworthy that the spleen, an organ in which there is hemoconcentration and presumably lowered oxygen tension in the sequestered red cells, was the initial site of infarction in persons with the sickle-cell trait.

The present observations demonstrate that metabolic acidosis may precipitate painful crises. During the induced crises, there was increased hemolysis, as evidenced by decreased hemoglobin levels and reticulocytosis. The numbers of "irreversibly sickled" cells in the peripheral blood also increased strikingly. The significance of the latter finding is not entirely clear, but the observations of Shen, Fleming, and Castle[17] indicate that red cells may assume the "irreversibly sickled" configuration after prolonged storage in the sickled form. Thus, the increased number of such forms in the acidotic patients probably reflects increased sequestration of the red cells in relatively hypoxic tissues. The evidence suggests, then, that acidosis induced greater susceptibility to sickling in vivo as well as in vitro.

The hazard of metabolic acidosis is probably greatest in young children, but it would seem wise to avoid mild acidosis even in persons with sickle-cell trait, particularly in patients with relatively high concentrations of S hemoglobin in their red cells.[13]†

Several observations indicate that red cells from a given patient may vary in their response to deoxygenation at different times.

† The sickle-cell trait is not entirely asymptomatic, as the aforementioned observations on high-altitude flyers indicate. We have observed depression of the hematocrits of two patients with sickle-cell trait who were undergoing anesthesia and one who was recovering from lobar pneumonia, with no apparent explanation other than changes in the susceptibility to sickling of red cells.

Storage at 37 C alters the viscosity curves, as does decreasing the temperature.[19] When patients with sickle-cell anemia were given adrenocortical hormones, some of them underwent hematologic improvement, and their red cells simultaneously became less susceptible than before treatment to the effects of partial deoxygenation on the degree of sickling.[20] Similarly, when crises were induced in certain of these patients by the administration of adrenocortical hormones, the viscosity curves shifted in the direction of increased susceptibility to partial deoxygenation.[21] We have observed transient spontaneous changes in the viscosity curves of patients with sickle-cell disease. Spontaneous crises have occurred as much as one month after the curve had shifted in the direction of increased susceptibility to deoxygenation. In one patient the curve shifted slowly in the direction of increased susceptibility to deoxygenation and, equally slowly, over the next two months returned to its former values; the patient remained asymptomatic. Occasional patients have been observed to undergo spontaneous crises with no shifts in their viscosity curves. No physiological bases for the spontaneous changes in susceptibility of the red cells of these patients has been determined. However, it is apparent that several different circumstances may produce painful crises and that local or systemic acidosis may be among these.

The administration of relatively large amounts of sodium bicarbonate intravenously caused decreased susceptibility to sickling. This was predicted from the study of the effects in vitro of changes in pH on the response to deoxygenation of cells containing S hemoglobin.[1,13] The administration of alkaline infusions also was associated with prompt symptomatic relief in some of the patients with painful crises. While these studies were in progress, Areu also reported that sodium bicarbonate provided symptomatic relief in children undergoing spontaneous sickle-cell crises.[22]

The management of crises is difficult. Crises are of variable duration and severity, and the patients have, after years of recurrent painful illness, tended to become apprehensive and dependent to such a degree that their subjective responses to therapy must be regarded critically. However, the prompt relief and the associated changes in the viscosity curves that followed the administration of alkali suggest that acute alkalosis may be of aid in the management of patients with sickle-cell crises.

The reasons for the failure of alkaline infusions in two cases are not clear. It is conceivable that acute alkalosis was not induced; objective measurements could not be performed in these two patients. It is also possible that the local intravascular changes become less readily reversed after a certain amount of damage has occurred. Further study of the efficacy of alkalinization in the management of painful crises is indicated in order to clarify the many questions raised by the present observations.

The maintenance of constant relative alkalosis in patients with sickle-cell anemia would appear to be desirable. The difficulties in doing so are well known, and our observations on the lack of effect of continued oral administration of sodium bicarbonate were therefore not unexpected.

The present data, like those obtained with use of adrenocortical hormones,[20] provide additional examples of the possibility of influencing the course of a genetically determined disease by manipulation of the environment in which the genetic abnormality functions. That extrahemoglobin factors other than the ones that have been studied play important roles in the pathophysiology of sickle-cell anemia is undoubted; studies directed toward the elucidation of such factors may provide further bases for the management of this disorder.

Summary

Because in vitro studies suggested that red cells containing S hemoglobin could be made more susceptible to the effects of deoxygenation by decreasing the pH of the blood, the effects of induced acidosis and

alkalosis in patients with sickle-cell disease were studied.

Metabolic acidosis was produced on two different occasions in a patient with sickle-cell anemia by continued administration of ammonium chloride and acetazolamide. The red cells of the patient became more susceptible to deoxygenation in vitro; there was evidence of increased hemolysis; increased numbers of circulating "irreversibly sickled" forms appeared, and typical painful crises occurred.

The intravenous administration of large amounts of sodium bicarbonate (3.3 mEq. per kilogram per hour) to patients with sickle-cell disease diminished the susceptibility to sickling of the red cells in vitro.

In six instances of painful crisis in patients with sickle-cell disease sodium bicarbonate was given in the aforementioned dose. Four of the crises responded promptly with relief of symptoms; there was no symptomatic response in the other two.

The prolonged oral administration of sodium bicarbonate did not cause alterations in the susceptibility to deoxygenation of the red cells of patients with sickle-cell disease, nor were there accompanying hematologic or biochemical changes to suggest that alkalosis had been induced.

Miss Geneva Daland; Mrs. Rose Blumenthal; Mrs. Joanne Norton; Mrs. Dorothy Conlon, R.N., head nurse of the Thorndike Ward; Dr. Charles V. Pryles, and the house staff of the Pediatric Service of the Boston City Hospital aided in these studies.

Dr. William B. Castle gave advice and made many contributions to this study.

Thorndike Memorial Laboratory, Boston City Hospital (30) (Dr. Kass).

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Exacerbation of Hyperthyroidism by Methimazole During Iodide Therapy

THADDEUS E. PROUT, M.D., and SAMUEL P. ASPER Jr., M.D., Baltimore

The care of patients with hyperthyroidism has been facilitated by the use of compounds which block the production of thyroid hormone. Early reports on the clinical use of such antithyroid drugs state that patients with thyrotoxicosis who were being treated with iodide frequently had an exacerbation of hyperthyroidism upon initiation of therapy with an antithyroid drug.[1,2] Although many other effects of these drugs on the thyroid have been studied,[3,4] this phenomenon has not been adequately explained. Further observations on the increase in the severity of symptoms which occur under these circumstances are recorded in this paper. Four patients with hyperthyroidism have been given methimazole during potassium iodide therapy. The effects of these medications on the symptoms, the values of the basal metabolic rate, and the levels of the butanol-extractable iodine of the blood in these patients are reported.

Method

The four patients in this study were women, ranging in age from 18 to 42 years. Each had a history of hyperthyroidism of three to six months in duration. The physical examination and laboratory tests also confirmed the diagnosis. The patients represented by Figures 1 and 4 were hospitalized during the period of study; the patients represented by Figures 2 and 3 were seen for evaluation three times weekly as outpatients. Determinations of the basal metabolic rate (BMR) were done in the rooms of the hospitalized patients. The outpatients rested in a cool quiet room for one hour prior to determination of the BMR. All tests were done in duplicate after the patient had fasted overnight. Butanol-extractable iodine (BEI) determinations were used to estimate the blood levels of thyroxin-iodine in the presence of the inorganic iodide administered during these studies.[5] Potassium iodide was prepared as a saturated solution. Methimazole and potassium iodide were administered orally.

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The Department of Medicine, The Johns Hopkins University School of Medicine. Fellow in Medicine (Dr. Prout); Associate Professor of Medicine (Dr. Asper).

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Results

The first patient (Fig. 1) was begun on potassium iodide, 5 drops three times daily. After four days of iodide therapy, methimazole, 15 mg. every eight hours, was initiated. There was a gradual decrease in the BEI and BMR throughout the period of study. No exacerbation of the hyperthyroidism occurred.

In the second patient (Fig. 2) potassium iodide, 20 drops three times daily, was administered for 21 days. There was a decrease in the BEI from an initial level of

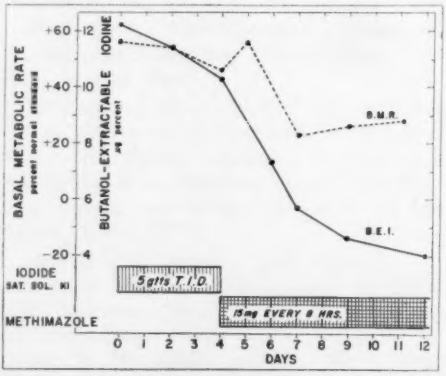

Fig. 1.—Serial determination of the BEI and BMR in a patient with hyperthyroidism given methimazole following iodide therapy. No exacerbation of the hyperthyroidism was seen when methimazole was begun.

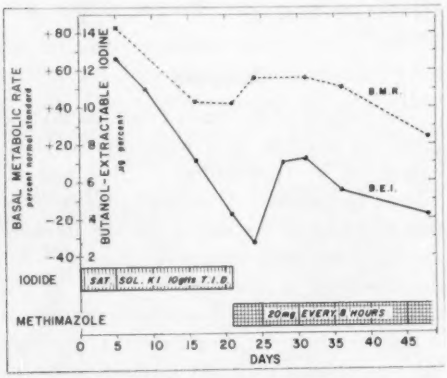

Fig. 2.—Serial determination of the BEI and BMR in a patient with hyperthyroidism given methimazole following iodide therapy. A decrease in the BEI and BMR was seen in this patient while she was being treated with potassium iodide. Following the substitution of methimazole there was a transient rise in the BEI and BMR.

12.6μg. per 100 cc. to a level of 2.7μg. per 100 cc. on the 24th day of therapy; this suggests that the iodide effect continued after daily administration was stopped. The change in the BMR is similar to that seen in the BEI. During the period of treatment with potassium iodide there was improvement in the patient's symptoms. Iodide was discontinued and methimazole, 20 mg. every eight hours, was begun on the 21st day. Between the 24th and 31st days of observation, there was an increase in the BEI to 7.2μg. per 100 cc. and a rise in the BMR. At the same time, the patient had a return of nervousness and heat intolerance. Methimazole was continued, and symptoms then abated as the BEI and BMR returned to normal.

This sequence of events was also seen in the third patient studied. As shown in Figure 3, there was a decrease in the BEI and BMR between the second and seventh days of iodide therapy. When the patient was seen on the seventh day, it was discovered that the potassium iodide had been discontinued because of the appearance of urticaria. It seemed unlikely that the urticaria was related to the potassium iodide, and the patient was encouraged to begin therapy again. On the 12th day of study both the BEI and BMR were elevated, but this was not accompanied by any detectable increase in symptoms. Subsequently the BEI and BMR decreased to 2.9μg. per 100 cc. and +31%, respectively, by the 19th day. At this time the iodide was discontinued and methimazole was started. Between the 24th and 30th days of study a rise in the BEI and BMR occurred and concomitantly, the patient complained of heat intolerance and mild diarrhea. With continued methimazole therapy the BEI and BMR returned to normal and the symptoms subsided.

Since iodide withdrawal of itself may be associated with an increase in the severity of hyperthyroidism,[6] a fourth patient was observed in whom treatment with iodide was continued after methimazole was added. This patient is represented by Figure 4. Despite severe symptoms the initial BMR was found to be only +31%. Following the initiation of potassium iodide treatment, the BMR increased to +76%. The possibility was considered that these results were spurious; the BEI determinations were reported later, however, as 4.4μg. and 10.9μg. per 100 cc., respectively, confirming the change in the BMR. During the period of iodide therapy there was a decrease in the

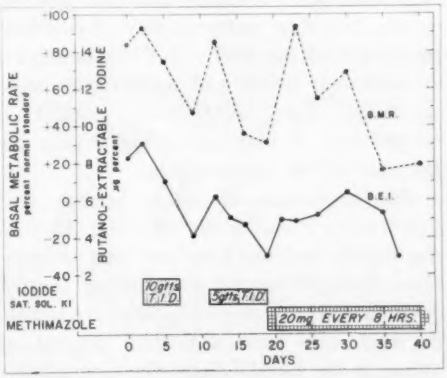

Fig. 3.—Serial determination of the BEI and BMR in a patient with hyperthyroidism given methimazole following iodide therapy. The BEI and BMR decreased in this patient during treatment with the potassium iodide. There was a temporary rise in the BEI and BMR when iodide was discontinued. The rise in the BEI and BMR, which occurred after substitution of methimazole, was prolonged.

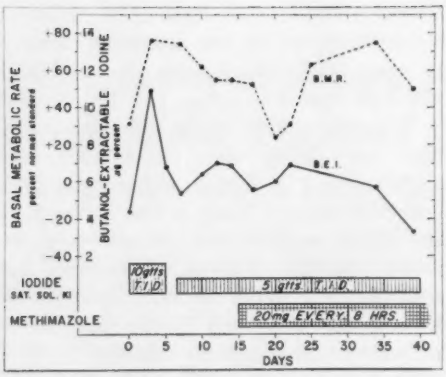

Fig. 4.—Serial determination of the BEI and BMR in a patient with hyperthyroidism given methimazole during iodide therapy. An increase in the BEI and BMR occurred in this patient when potassium iodide was initiated. There was a subsequent decrease in the values of the tests on continued therapy. When methimazole was added there was a return of symptoms and a rise in the BMR, despite the fact that iodide was continued.

BEI and BMR. Potassium iodide was continued, and methimazole, 20 mg. every eight hours, was begun. The BMR, which had continued to decrease for several days after methimazole was started, subsequently increased to +63% and +70% on the 25th and 34th days, respectively. There was a return of nervousness and palpitation simultaneously with the rise in the BMR. A determination of the BEI was not obtained on the 25th day, unfortunately. Symptoms subsided, and the BEI and BMR decreased as potassium iodide and methimazole were continued. Thus, methimazole induced an exacerbation of hyperthyroidism despite the fact that iodide was continued.

The institution of iodide therapy was followed by a rise in the BEI and BMR in the fourth patient. Two previous observations shed light on this phenomenon. Firstly, patients with thyrotoxicosis who are iodine-depleted may have a normal content of iodine in the blood.[7] Secondly, Graves' disease is occasionally exacerbated by iodide therapy.[8] The increase in circulating thyroxin seen in this patient is similar to the phenomenon of iodine-induced hyperthyroidism (jodbasedow) in man[9] and to the reactions following iodine administration to animals with goiters due to iodine deficiency.[10,11]

Comment

The release of thyroxin from the thyroid gland of patients with hyperthyroidism is stimulated by thyrotropin (TSH) and inhibited by iodide.[12] Attempts to relate these two phenomena through a specific mechanism have not been successful. Loeser[13] has demonstrated a decrease in TSH in the pituitary following iodine administration. It is unlikely, however, that there is physiological suppression of TSH production by iodine, since iodine has no effect on thyroxin release from the normal thyroid gland.[14] DeRobertis[15] has reported the presence of a proteolytic enzyme in extracts of the thyroid gland which was increased by prior administration of TSH and decreased by iodine therapy. He postulated that this enzyme was important in the release of thyroxin from thyroglobulin. The proteolytic enzyme is inactivated by iodine and may prevent thyroxin release by this means.[16] On the other hand, Astwood states that it is unlikely that this vigorous chemical reaction would take place in the thyroid gland.[3]

Others workers have postulated that iodine inhibits the action of TSH by a direct effect on circulating TSH. Albert and his co-workers[17] have demonstrated the inactivation of TSH by iodine in vitro. It has also been shown that the discharge of radioiodine from the thyroid gland which is stimulated by exogenous TSH is partially inhibited by the administration of iodide.[12,18] This inhibition can be overcome by the administration of additional TSH.[16] Hence, the discharge of thyroxin from the thyroid can be stimulated despite iodide suppression either by increasing the effect of the TSH or by decreasing the effect of the iodide. Methimazole may have induced thyroxin release in the patients studied here by either of these two general mechanisms. The antithyroid drugs may enhance the effect of TSH,[19] reactivate TSH that has been inactivated by iodine,[20] or react with iodine directly.[3]

Patients with thyrotoxicosis who are treated simultaneously with iodide and an antithyroid drug may have an exacerbation of the disease when iodide is withdrawn similar to that seen in the patients reported here.[21] The therapy of hyperthyroidism with iodide alone results in a more rapid improvement than is seen when only an antithyroid compound is used. Withdrawal of the iodide, during therapy with both the antithyroid compound and iodide, is said to allow the gland to escape to the level of control established by the antithyroid compound alone.[21] This sequence has not been studied; in the fourth patient, however, the rise in the BMR and return of symptoms occurred when methimazole was added, despite the fact that iodide was continued.

Iodide appears to block thyroxin release effectively in hyperthyroid patients,[12,18] although thyroxin production continues. This fact has been offered as an explanation for thyroid crisis following iodide withdrawal[16] and for the return of symptoms sometimes seen during treatment of Graves' disease with iodide alone.[22] Methimazole induced the release of thyroid hormone in the patients in this study, whether iodide was continued or not, provided that the iodide had been given for a sufficient length of time. The exacerbation that ensued was mild and transient and was maximal from 9 to 14 days after the initiation of methimazole administration. When TSH is given to patients with thyrotoxicosis under therapy with iodide the stimulation of the thyroid occurs within 24 hours.[14] This suggests that the release of thyrotropin induced by methimazole in the patients reported here did not result from the reactivation of inactive thyrotropin already present. The data available are insufficient to establish the validity of any of the alternative mechanisms of action which have been cited.

Summary

Four patients with a history of hyperthyroidism of recent onset were given potassium iodide followed by methimazole. An initial response to iodide therapy was obtained, as evidenced by an amelioration of symptoms and by a decrease in the values of the basal metabolic rate and the levels of butanol-extractable iodine in the blood. Following the addition of methimazole there was, in three patients, a transient exacerbation of the hyperthyroidism whether or not iodide was continued. Methimazole caused the increase in these symptoms by inducing the release of thyroxin. The time interval between the administration of methimazole and the maximal effect of the thyroxin suggests that the release of thyroxin depends upon an action of methimazole other than its reactivation of thyrotropin already present.

The Johns Hopkins Hospital (5).

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Recurrence of Hemorrhage from Medically Treated Gastric Ulcers

Four- to Eight-Year Follow-Up of Forty-Seven Patients

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The frequency of recurrence of gastric ulcer during medical management has been reported by several investigators to range from 40% to 85% in patients followed for five years.[1-8] Few data are available concerning the frequency of recurrent hemorrhage from gastric ulcer. Swynnerton and Tanner [3] reported that 139 of 498 selected patients followed for 5 to 12 years had hematemesis or melena during the course of the disease. Flood and Hennig [4] reported gross hemorrhage in 38 of 101 patients, and 13 of these (33%) bled again within the 1- to 12-year follow-up period.

Of 47 patients seen at the Boston City Hospital who bled from a benign gastric ulcer and were treated medically, only 3 bled again during the following four to eight years. Included in this study are nine patients who refused gastric surgery when it was recommended. This low incidence of recurrent bleeding differed so markedly from previous reports that a detailed review seemed indicated.

Clinical Material and Method of Selection

From November, 1947, to January, 1952, one hundred nineteen patients admitted to the Second and Fourth Medical (Harvard) Services at the Boston City Hospital because of gastrointestinal hemorrhage had one or more gastric ulcers either demonstrated by emergency radiographic study or seen at gastroscopy.

The method of management of these patients has been previously reported.[10] Surgical consultation was requested in 89 patients within 48 hours after admission, in keeping with the combined medical-surgical-radiological team approach to the management of gastrointestinal hemorrhage. A Sippy type of diet, usually begun soon after admission, progressed eventually to full bland diet, usually within two weeks. Decisions regarding surgery were made in consultation with the house and visiting medical and surgical staffs. Forty-nine patients (41%) required gastric surgery during the initial hospitalization to control the hemorrhage. Gastric carcinoma was found in four of these patients. Eleven patients (9%) died while receiving medical treatment. Three other patients had coexisting active duodenal ulcers, and two more had demonstrable esophageal varices.

Repeat upper gastrointestinal x-ray studies and/or gastroscopy were performed in 45 patients, and the ulcer was believed to have healed completely in 40. The shortest time in which healing was observed was 10 days; the longest was 5 weeks. Roentgenographic healing was felt to have been inadequate in five patients. One of these refused surgery. Gastric carcinoma was found at surgical

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exploration in another patient; the three remaining patients had chronic benign gastric ulcers histologically.

Thus, 69 patients were excluded from the study because of (1) death, (2) presence of bleeding sites in addition to or other than gastric ulcer, or (3) gastric surgery. The remaining 50 patients (42%) were treated medically and thus were available for this study. There were 31 men and 19 women in the group, whose ages ranged from 26 to 86 years; 18 patients (36%) were over 65 years of age. Thirty-seven patients (74%) had experienced ulcer-type symptoms for one to five years; thirteen (26%) denied such symptoms prior to gastrointestinal hemorrhage. Forty-four (88%) of the patients had melena, hematemesis, or both on admission to the hospital; six gave histories of melena or hematemesis and had chronic blood-loss anemia. Nineteen (38%) were admitted with signs of shock. Twenty-nine received an average of 3.6 transfusions, with a range of 1 to 12 units of whole blood. According to Smythe's[8] definition of severity of hemorrhage, 19 patients (38%) had "minimal hemorrhage" (hematocrit remained above 35% and patient was not transfused), 15 patients (30%) had "moderate hemorrhage" (hematocrit remained above 25% and the patient received less than 2500 cc. of blood), and 10 patients (20%) experienced "severe hemorrhage" (hematocrit fell to 25% or below and the patient received 2500 cc. or more of blood). In the last group are included four of the patients who refused gastric surgery.

Previous gastrointestinal hemorrhage had been experienced by four patients. One bled from a duodenal ulcer; another bled from "hemorrhagic gastritis" and superficial ulcerations which were seen gastroscopically; the two others were not studied at the time of hemorrhage.

As a group, the patients came from the poorer sections of the city. Serious alcoholism was admitted by more than one-half of the patients. Thirty-seven patients (74%) had significant coexisting medical disease including marked alcoholism (Table 1).

TABLE 1.—*Coexisting Medical Diseases in Fifty Patients Treated Medically for Bleeding Gastric Ulcer, 1948-1952*

Significant Medical Diseases	Patients, No.*
Azotemia with chronic nephritis	2
Severe bronchiectasis and/or emphysema	5
Cirrhosis	4
Acute fatty liver	7
Asymptomatic rheumatic or hypertensive heart disease	10
Congestive heart failure	5
Previous myocardial infarction	6
Angina pectoris	3
Severe peripheral arterial insufficiency	2
Poorly controlled diabetes mellitus	3
Primary cerebellar atrophy	1
Acute and/or chronic alcoholism	26

* Twenty-four patients had more than one of the diseases listed.

One or more gastric ulcers were demonstrated by radiography alone in 25 patients, by gastroscopy and radiography in 12 patients, and by gastroscopy alone in 13 patients after an emergency upper gastrointestinal x-ray study was not diagnostic. In this group the gastroscopic description* was "single superficial ulcer" in six patients, "more than one discrete superficial ulcer" in four patients, and merely "gastric ulcer" in three patients.

Nine patients rejected surgery when it was recommended because of (1) severity of the hemorrhage in five otherwise healthy patients, (2) recurrent active bleeding during hospitalization in two patients, and (3) persistent occult bleeding from an incompletely healed gastric ulcer in two patients. The clinical data concerning these patients are presented in Table 2.

Method and Results of Follow-Up (Table 3)

Routine management after discharge was maintained by 35 patients; 25 regularly visited the Gastrointestinal Outpatient Clinic, and 10 were followed by private physicians. Fifteen patients (30%) did not have any regularly administered medical care from the time of discharge until the time this study was made four to eight years later. Data were obtained on 47 (94%) of these patients (Table 2). Thirty-four patients were available for examination and diagnostic studies; 12 patients had died, and autopsy data were available for 9 of these. One patient was contacted by mail. In addition, interval data in some cases were obtained from subsequent hospital admissions, from private physicians, and from patients' families. Three patients could not be contacted.

Fourteen patients remained free from apparent gastric ulcer recurrence, among them were four who died of diseases other than gastric ulcer. Twenty-four patients had one or more recurrent gastric ulcers, including three who died of diseases other than gastric ulcer. Gastric ulcer complications developed in seven patients, necessitating gastric re-

* Gastroscopic examinations were performed by Dr. I. R. Jankelson, Chief of the Gastrointestinal Clinic, and Dr. Charles McClure, Boston City Hospital.

TABLE 2.—*Clinical Data Concerning Nine Patients with Bleeding Gastric Ulcers Who Refused Surgery, 1948-1952*

Case	Age, Yr.	Sex	Date	Past History	Alcoholism	Present Illness	Significant Physical Findings	Laboratory Data			
								Hct.	Stool	Gastric Analysis	Other
1	72	M	3/50	Unremarkable	Infrequent	5 days nausea, weakness, tarry diarrhea	Slight shock; B. P. 100/60; pulse 130; emphysema	16	Black tarry	Not performed	NPN 89 mg., PT 60%
2	63	M	8/49	Unremarkable	None	4 days melena; 2 days hematemesis, weakness	None	30	Black tarry	24 u. free acid	
3	58	M	2/48	Myocardial infarction, 2 yr. previously; ulcer symptoms, intermittently, 3 yr. (? gastric ulcer on x-ray)	None	Massive hematemesis, melena with syncope	Shock, epigastric tenderness; B. P. 90/50; pulse 110	25	Black tarry	45 u. free acid	
4	66	M	3/49	Rare ulcer symptoms, 2 yr; primary cerebellar atrophy	Infrequent	Epigastric pain; 10 lb. weight loss, tarry stool 1 day, & marked weakness	None	27	Black tarry	Free acid present	Gastric cytology neg. twice
5	49	M	4/49	Known compensated mitral stenosis; no prior ulcer symptoms	Infrequent	Sudden massive hematemesis and syncope	Shock; B. P. 90/60, pulse 100; mitral stenosis	27	Black tarry	12 u. free acid	
6	59	M	4/50	Ulcer symptoms at 2 and 5 yr. of age	Moderate	Hematemesis, weakness, and dizziness, 2 days duration	Epigastric tenderness, enlarged liver	22	Black	Free acid present	
7	44	F	12/48	10 yr. of intermittent ulcer symptoms	None	Tarry stools, 1 wk. duration, with weakness & syncope	Sweating and pale, B. P. 110/60	21	Black	5 u. free acid	
8	53	M	10/48	6 yr. of intermittent ulcer symptoms; hematemesis, 2 yr. previously	Moderate	Hematemesis, melena and syncope, 2 days duration	Shock, pale; B. P. 95/50	27	Not noted	20 u. free acid	
9	53	M	6/50	Infrequent ulcer symptoms, 3 yr. moderately severe rheumatoid arthritis, inactive	Infrequent	6 wk. epigastric pain; 1 wk. melena; 2 days hematemesis & weakness	Kyphosis, rheumatoid arthritic deformity of hands and feet; normal B. P. and pulse	32	Tarry	Not performed	

TABLE 2.—*Clinical Data Concerning Nine Patients with Bleeding Gastric Ulcers Who Refused Surgery, 1948-1952*—Continued

Case	Age, Yr.	Sex	Date	Roentgen Diagnosis	Gastroscopic Diagnosis	Blood Transfusions, Cc.	Course, Indication for Surgery
1	72	M	3/50	Prepyloric lesser curvature ulcer, appears benign; rugae	Not performed	2,500	Stabilized vital signs and Hct. at 37 by second day; recurrent black tarry stools on 10th hospital day with nausea and blood aspirated from stomach; vital signs remained stable
2	63	M	8/49	Lesser curvature midbody ulcer, ? benign	Benign gastric ulcer	2,000	Stools intermittently guaiac-positive for 4 wk. & gastric ulcer had not completely healed; surgery recommended because of continued oozing and suspicion of gastric malignancy
3	58	M	2/48	2 prepyloric ulcers on lesser curvature	1 gastric ulcer seen, benign	2,500	Stabilized vital signs within 9 hr.; surgery recommended because of age and massive hematemesis
4	66	M	3/49	Lesser curvature midbody ulcer, large and appears benign	Benign gastric ulcer	500	Intermittent guaiac-positive stools for 2½ wk.; surgery suggested because of continued bleeding from a gastric ulcer which had not completely healed, suspicion of carcinoma
5	49	M	4/49	Lesser curvature antral ulcer, ? benign	Benign gastric ulcer	1,500	Stabilized vital signs within 7 hr.; surgery recommended because of age and sudden hematemesis in previously asymptomatic gastric ulcer patient
6	59	M	4/50	Posterior wall, large ulcer, ? malignant	Benign gastric ulcer	2,500	Stabilized vital signs promptly after admission; surgery advised as prophylaxis against future bleeding
7	44	F	12/48	Lesser curvature midbody ulcer, ? benign	Healing gastric ulcer (23d day)	3,000	Stabilized vital signs within 12 hr.; surgery advised because of massive hematemesis and patient's age
8	53	M	10/48	Lesser curvature midbody ulcer, penetrating, ? malignant	Multiple superficial gastric ulcerations	1,500	Stabilized within 8 hr.; surgery suggested because of previous bleeding and because patient had not been taking care of himself
9	53	M	6/50	Prepyloric ulcer, lesser curvature, ? benign	Refused	500	Stable vital signs on entry; recurrent guaiac-positive stools on 9th day with nausea and blood aspirated from stomach; surgery suggested because of recurrent bleeding with a gastric ulcer still present

TABLE 3.—*Summary of Follow-Up Data Concerning Forty-Seven Patients with Benign Gastric Ulcer Hemorrhage, 1947-1952*

I. Survival

35 patients alive (including 4 of the patients who had refused gastric surgery)
12 patients died (none from gastric ulcer hemorrhage)

No. of Yr. Initial Hosp.	From Refused Surgery	Diagnosis	Autopsy
1	No	Myocardial infarction	Yes
1	No	Diabetes mellitus, pneumonia	Yes
2	No	Cerebral vascular accident	No
3	Yes	Chronic glomerulonephritis	Yes
3	No	Sepsis	No
3	No	Carcinoma of rectosigmoid	Yes
4	Yes	Mesenteric infarction, preoperative	Yes
5	Yes	Congestive heart failure	No
5	No	Cirrhosis, diabetes mellitus	Yes
6	Yes	Myocardial infarction	Yes
7	Yes	Subacute bacterial endocarditis	Yes
8	No	Cerebral vascular accident	Yes

II. Gastric ulcer recurrence	Patients, No.
1. One or more recurrences, 30 patients (64%)	
Ulcer symptoms with x-ray demonstration of one or more ulcers	14
Ulcer symptoms with normal x-ray study	4
Ulcer symptoms without x-ray study performed	9
No ulcer symptoms but x-ray demonstration of one or more ulcers	3
2. Neither ulcer symptoms nor x-ray recurrence	17(36%)

III. Gastric ulcer complications, 8 patients (17%)

1. Perforation and/or obstruction
   3 patients required surgery for treatment of these complications 1, 3, & 5 years after the initial hospitalization
2. Intractability
   2 patients required gastric surgery 1 and 5 yr., respectively, later because of persistent ulcer pain; in 1 case a penetrating gastric ulcer was discovered at operation
3. Hemorrhage
   1 patient developed a recurrent gastric ulcer (prepyloric) 3 times, with hematemesis on each occasion and continued to refuse surgery
   2 other patients had hematemesis and melena from recurrent gastric ulcers and did not require surgery

section in five for penetration, perforation, obstruction, or intractability. The remaining two experienced hemorrhage from a gastric ulcer but did not require surgery.

The clinical courses of the nine patients who refused gastric surgery are summarized in Table 2 and in the Appendix. Of four living patients, one was asymptomatic. Two had symptoms but no evidence of recurrence by x-ray. The fourth patient had four more bleeding episodes and continued to refuse surgery. Five patients died. Gastric ulcer recurrence was demonstrated in three patients and contributed to death in one (Case 2, Appendix). No further hemorrhage occurred in eight of the nine patients.

Each of the 34 patients available for examination was studied for the presence

of gastric cancer by physical examination, upper gastrointestinal roentgenographic study, and gastric cytology. Gastroscopy was performed in seven patients when x-rays demonstrated questionable gastric lesions. Gastric carcinoma was not evident in any of these nor in the 12 patients who died, 9 of whom came to autopsy.

Comment

Only 3 of the 47 patients (6%) experienced recurrent gastric ulcer hemorrhage during the 4- to 8-year follow-up period. Previous investigators have reported recurrence of hemorrhage from gastric ulcer in 16% to 33% of patients followed from 1 to 12 years.[1-8] It does not seem likely that variations in hospital populations alone account for the differences between these studies and our own; because of the high incidence of alcoholism and coexistent medical diseases, one might have expected a high frequency of recurrent hemorrhage in our patients. The manner of selection may contribute to the apparent difference. In the studies of Swynnerton and Tanner[3] and Flood and Hennig[4] this is not clear. In the latter study only those patients who remained under close observation for at least one year were included. How many well patients left the clinic during that year is not stated. The present study, on the other hand, includes all patients with bleeding gastric ulcers who were treated medically from 1948 to 1952. At least 15 patients were not seen professionally after leaving the hospital until they were contacted as part of this study; 13 of these had remained symptom-free.

Since 50% of the patients studied were alcoholics, the reliability of their follow-up histories may be questioned. Some of the patients may have experienced further gastrointestinal hemorrhage and yet denied this when questioned by the examiners; smaller amounts of bleeding may have been overlooked. However, the fact remains that regardless of continued alcoholism these patients did not experience bleeding severe enough to require hospitalization, and none died of recurrent hemorrhage.

It is important to emphasize that early roentgen examination was routinely performed on our patients with massive hemorrhage during the period of this study. We have learned that this method may pick up gastric ulcers which may be radiologically not apparent when repeat examination is performed in as few as seven days.[14] This may account in part for the difference between the patients in our study and those in Banks and Zetzel's series[5] in which emergency roentgen examination was not practiced and only patients with radiologically unequivocal lesions were accepted. Furthermore, the lesion in 15 of the 50 cases was seen on gastroscopic examination performed because an emergency gastrointestinal x-ray examination failed to reveal a definite lesion. In 11 patients the findings were described as "single or multiple discrete superficial gastric ulcers"; nine of these patients had acute and/or chronic alcoholism. None of these 15 patients had recurrence of gastric hemorrhage; in 3, gastric ulcers were seen at x-ray examination at some time during the follow-up period, and one of the three required surgery for perforation. No symptoms distinguished this group from the remainder of the patients. It is not possible to state whether this type of ulceration has the same pathogenesis as chronic gastric ulcer; its tendency to recurrence and complications has not been carefully studied.

The high recurrence rate of gastric ulcer symptoms of 40% to 85% within five years in most large series[1-8] is well established and is confirmed by the recurrence rate of 64% in this series of patients.

With the exception of patients who refused surgery, virtually all patients with uncontrollable hemorrhage or recurrent hemorrhage in the hospital were operated on during the initial hospitalization. This amounted to 43% of the total group of 116 patients. In another study on this service 25% of 79 patients with bleeding duodenal

ulcer required surgery.[11] These data support the observation that bleeding gastric ulcers are more likely to require surgery for control of hemorrhage than bleeding duodenal ulcers.[12]

Of the nine patients who refused surgery against advice, only one had recurrent gastric hemorrhage and none died. Three patients stopped their heavy intake of alcohol after leaving the hospital and maintained satisfactory follow-up. Five patients did not suffer any recurrence of gastric ulcer symptoms, surviving three to seven years before dying of other medical diseases which were present at the time surgery was recommended. In other studies of medically treated gastric ulcer the incidence of subsequent operative intervention for all causes has varied from 10% to 50%.[5] Only six (13%) of the patients in the present series required operative intervention.

Several investigators have commented on the frequency of serious complications in benign gastric ulcer and the severity of recurrent gastric ulcer.[5,13] Banks and Zetzel observed that only 11 of the 48 patients remained symptom-free during a 7- to 15-year follow-up period. In the present series the symptoms of recurrence were no more severe than those of the initial illness and gastric resection was performed on only six patients because of complications (Table 3).

In 11 patients a recurrent ulcer was demonstrated in the same area of the stomach; in 6 patients the recurrent ulcer was in a different area of the stomach, and in 4 patients no ulcer could be visualized by x-ray or gastroscopy, although the symptoms were considered typical of an active ulcer. In three patients a gastric ulcer was seen on x-ray study when the patients were completely symptom-free. Flood and Hennig [4] reported in their series of 101 patients with gastric ulcer 6 with an active ulcer crater seen on x-ray in patients who had had no recent ulcer symptoms. Hence, the number of recurrent gastric ulcers in any series of patients may be, in fact, higher than that reported in symptomatic patients alone.

Gastric cancer was not demonstrated in any of the patients followed. This is probably due to the fact that gastric cancer is an infrequent cause of massive upper gastrointestinal hemorrhage and patients with bleeding gastric carcinoma usually come to surgery promptly.

Despite the distressing frequency of recurrence of symptoms in gastric ulcer, one cannot conclude that gastrectomy is imperative in order to prevent further hemorrhage in all patients who have recovered from bleeding. The fear of sudden and unpredictable fatal bleeding is often evoked to justify a decision for prompt elective gastrectomy. We found little cause for such fear. Certainly, it is no substitute for careful deliberation in deciding for or against surgery, particularly in the older patients, to whom gastrectomy is both painful and dangerous.

Summary and Conclusions

Forty-seven patients treated medically for bleeding gastric ulcer were reviewed four to eight years after the bleeding episode; nine of these patients had refused surgery at the time of their initial hospitalization. Hemorrhage recurred in only three patients; penetration, perforation, obstruction, or intractability, in six patients. There were no cases of gastric carcinoma found at follow-up. The over-all recurrence rate of all symptoms was 64%.

The view that the hazard of recurrent hemorrhage makes surgery mandatory for all patients who have recovered from benign gastric ulcer hemorrhage is not supported by this study. This is especially true in patients in the older age group.

Early roentgen diagnosis and gastroscopy are necessary for satisfactory diagnosis of many gastric ulcers, which may not be apparent when examination is delayed.

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Appendix: Follow-Up of Patients Refusing Gastric Surgery, 1948-1952

CASE 1.—Readmitted with epistaxis 27 months after discharge; no interval ulcer symptoms but upper gastrointestinal x-ray series demonstrated lesser curvature gastric ulcer. Renal status was unchanged, with NPN 89 mg. per 100 cc., isosthenuria, and hypertension (B. P. 190/100); ulcer healed by x-ray in three weeks. Twelve months later ulcer symptoms recurred and upper gastrointestinal x-ray study was normal. Patient died four years after initial hospitalization, with uremia. Autopsy revealed chronic glomerulonephritis, with no evidence of a gastric lesion.

CASE 2.—Gastric ulcer healed as shown on upper gastrointestinal x-ray study one month after discharge. Patient did well until one and one-half years later, when acute perforation of lesser curvature gastric ulcer occurred and was closed; biopsy revealed "chronic gastric ulcer"; upper gastrointestinal x-ray series on 20th hospital day demonstrated small lesser curvature antral ulcer; reexploration revealed subdiaphragmatic abscess, and therefore gastrectomy was not performed. Patient did well clinically and ulcer healed as shown by follow-up x-ray study two months later. He remained symptom-free for 26 months and then was admitted to another hospital with pyloric obstruction. While being prepared for surgery he suddenly went into shock and died. Autopsy revealed infarction of the large bowel and segmental areas of the small bowel as well as a large lesser curvature antral chronic gastric ulcer which penetrated into the pancreas.

CASE 3.—Infrequent upper abdominal postprandial distress was noted, but there was no radiographic recurrence of gastric ulcer on several x-ray studies. He was readmitted to the hospital five years later for epistaxis and moderately severe iron-deficiency anemia; an upper gastrointestinal x-ray study revealed irregularity of the lesser curvature due either to small gastric ulceration or to early carcinoma; two weeks later repeat x-ray study was normal, as was gastroscopy. Patient died at home in congestive heart failure eight months later, six years after his initial hospitalization. No autopsy was performed.

CASE 4.—Patient remained symptom-free with ulcer healed by x-ray with infrequent pyrosis. Four years later he was found to have esophageal carcinoma with partial obstruction; a Wookey procedure was performed. Two years later he is doing poorly, although free of ulcer symptoms.

CASE 5.—On strict ulcer diet patient did well, without symptoms; however, interval x-ray study six months later, after discharge, demonstrated a smaller lesser curvature ulcer which was visualized gastroscopically and thought to have been benign; repeat x-ray study in two months was normal. Patient remained symptom-free and seven years later died of subacute bacterial endocarditis with cerebral embolization. Autopsy did not reveal any gastrointestinal pathology.

CASE 6.—Patient followed dietary suggestions and made frequent visits to the gastrointestinal

clinic for check-up; there was neither symptomatic nor radiographic recurrence. Six years later he died of a massive myocardial infarction. Autopsy did not reveal any gastric or duodenal pathology.

CASE 7.—Previous ulcer symptoms recurred approximately twice a year and responded to medical management. Numerous upper gastrointestinal tract x-ray studies and two gastroscopic examinations did not reveal any further evidence of gastric or duodenal pathology.

CASE 8.—Patient has done well except for occasional symptoms of ulcer disease without radiographic evidence of a recurrent gastric ulcer three years after discharge from the hospital.

CASE 9.—Gastric ulcer healed during hospitalization, and patient remained symptom-free for six months, at which time hematemesis recurred and a prepyloric ulcer was demonstrated; surgery was refused. He experienced hematemesis again 6 months and 18 months later and on each occasion refused surgery. For the past three years he has not bled but has experienced intermittent ulcer symptoms. Upper gastrointestinal x-ray series six years after initial hospitalization did not show any abnormality.

Differences Between Smokers and Nonsmokers

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Why some men smoke and others do not has never been adequately explained. The question has some medical relevance now that heavy smoking has been shown to have relationship to lung cancer and possibly to coronary disease and since there have been certain therapeutic indications for the control of smoking. It is at least theoretically possible that smoking is often a symptom or sign of physiologic or personality characteristics which themselves may influence disease. The opportunity has recently come to explore some data already collected in respect to differences of physique, physiology, and personality between smokers and nonsmokers. This is a part of a larger study of smoking habits. Charles C. McArthur, Ph.D., is preparing material from the same source on the psychodynamics of smoking,[1] and Carl C. Seltzer, Ph.D., on physique of smokers.

The group under study comprises 252 college graduates, now in the age range of 33 to 37 years, most of them married and settled in a wide variety of occupations throughout the country. They are members of the Study of Adult Development (Grant Study),[2] which is a long-term study of selected "normal" college men, who were first seen in 1938 to 1942 and have been pursued by interview and questionnaire to the present. During the gathering of the data, inquiry into smoking habits was given the same degree of attention as other aspects of information obtained about these participants.

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I. The Collection of Data on Smoking

The original group of participants comprised 268 men. Ten of these left the study during the initial years, and six were killed during the war, leaving two hundred fifty-two men. Three additional men had not replied to questionnaires sufficiently to be included, and one more was excluded from the group by the nature of his selection and by personality difficulties. However, four referred cases were included. These were men who had been studied in precisely similar ways, whose subsequent careers have shown good accomplishment, and who have been faithful in follow-up questionnaires. For this total group of 252 men the annual questionnaires have been returned by 80% on the average for each year. Ninety per cent of the men have returned at least one questionnaire every two years.

During the initial medical examination in 1938-1942 the smoking habits, among other habits, were recorded by the following tabular scheme: Tobacco—none, daily, varies; cigarettes—0, 1-5, 6-10, 11-19, 1 pack, 2+ packs per day; pipes—0-1-2-3-4-5+ per day; cigars—0-1-2-3-4-5 per day. In addition, the psychiatrists and others recorded for many cases the participants' comments about their smoking habits. Each full questionnaire in years subsequent to World War II has contained the above tabular scheme under a section on health and habits.

Table 1 shows the number of men answering the tobacco question each year and the per cent of men under each category of smoking. The year 1945 is omitted because of too few responses. This was the last year of the war and was preceded by a questionnaire and immediately followed by a thorough questionnaire concerned with activities during the war.

TABLE 1.—*Summary of Data on Smoking Habits*

Year	Men Answering Questionnaire, No.	Cigarettes Daily *			Pipes, 1 or More Daily *	Cigars, 1 or More Daily *
		None	1–19	1 Package or More		
1938–1942	251	50.2	42.3	7.6	0.8	15.6
1943	108	42.6	38.9	18.5	5.5	15.8
1944	110	48.2	22.8	29.0	2.7	19.1
1946	173	47.4	29.0	23.7	3.5	19.6
1947	153	46.4	25.5	28.1	2.7	18.3
1948	145	44.2	20.7	35.2	0.7	15.8
1949	195	46.7	24.1	29.2	3.1	20.0
1950	202	46.5	21.2	32.2	4.0	19.8
1951	202	45.0	21.8	33.2	4.5	15.8
1952	203	51.2	18.8	30.0	4.0	14.8
1953	177	51.4	19.2	29.3	2.3	12.4

* Numbers indicate per cent of men.

The nonsmokers varied between 44.2% and 51.4%, the largest numbers falling during college and in recent years. When one considers the history of smoking over the whole period of some 15 years, the numbers of nonsmokers are considerably reduced because of those who took up smoking tentatively or intermittently. Only 51 men have never smoked (20%). The table shows an increase of heavier smokers (one package or more daily) to a peak of 35% in 1948 and then a diminution of numbers to 29% in recent years. Smoking during college in 1938 to 1942 was, for many men, merely an intermittent experiment, and the figures at this time should be discounted. Relatively few men smoked two or more packages of cigarettes a day, not more than four men prior to 1950. Eleven men smoked two or more packages a day in 1952.

Pipe or cigar smoking was less frequent than cigarette smoking. Cigar and pipe smoking will be essentially disregarded in the present study.

A threefold classification of smoking has been constructed, based upon long-term observation of each subject. The men who never smoked are grouped with 10 men who smoked rarely and intermittently only before 1947, and they are considered nonsmokers. They total 61 men, or 24.2%. A group of moderate smokers, 95 men, or 37.7%, includes all of those smoking up to 19 cigarettes daily and also 30 men who were chiefly cigar or pipe smokers. The remainder, called heavier smokers, totalling 96 men, or 38.1%, smoked one or more packages of cigarettes daily, either continuously or for a majority of the years during which they were followed.

II. Personality Differences

Case Descriptions

The records of the Study of Adult Development are a rich source for case description. Verbal descriptions of persons can supply ample evidence of personality differences without lending themselves to any easy statistical handling or proof. A reading of records showed marked differences between some of the smokers and the nonsmokers. The following brief case descriptions, somewhat disguised, illustrate this. The first five cases are the men who smoked most heavily (two or more packages of cigarettes a day for a prolonged period of time).

Five Heaviest Smokers

CASE 1.—A product of a poor family, with dominating critical parents, he had to work his way through college. He chose a dangerous branch of the armed service during the war, one for which he had admitted some fear, and fulfilled his duties well. Active physical work for a large firm which sets standards for employees but provides housing, pension, and insurance plans. Some marital difficulties. Somewhat suspicious of others.

CASE 2.—Hard-driving and striving worker. Remarkable combat record during the war, including long periods of dangerous fighting and several wounds. Psychosomatic symptoms on returning to civilian life. A stormy courtship, but eventually a successful marriage and successful career. Aggressive worker in a highly competitive business field.

CASE 3.—This man was cast off by a broken family at an early age to make his own way.

Highly intelligent, bitter, critical, he has met one setback after another, usually of his own making. Difficulty fitting into conventional social life; many love affairs. Difficulty controlling drinking. Has at last found a satisfactory career at which he works with almost desperate energy.

CASE 4.—A robust energetic headstrong fellow, who broke from his conservative family while in college, got into brawls, performed impulsive acts, and in general was a problem to family and college. The psychiatrist called him an "irregular and unsystematic boy." In his junior year he had a "rearrangement of ideas" and became a good student. He was commander of a combat unit of 250 men during the war. Since then he has been an energetic advocate of social reforms and actively puts his ideas into effect. Moderate marriage difficulties.

CASE 5.—In college described as a "good-looking, active, energetic, athletic boy." One of the few with tattoo marks. His mother said, "He gives birth to a new idea and falls in love with a new girl every day." During the war was a naval officer in combat. His work requires travel in foreign countries and considerable initiative and physical activity. Marriage resulted in divorce.

There is no question that these five men have something in common. They show energy, seeking for danger, restlessness, and a kind of independence which keeps them actively engaged in some enterprise which appeals. They are interesting men who perform useful work. They seem to be well sexed, but they have had difficulties with marriage, perhaps stemming from their independent natures. Their smoking to excess seems to be a symptom of their great restlessness.

A random sample of 5 of the 51 nonsmokers was taken, and their brief case summaries are as follows:

Five Nonsmokers

CASE 6.—Obtained perhaps the highest Army α grades of the study. Public school and Middle West background. Parents both died when he was young, and he was raised by an older sister who gave him good security under the circumstances. He reacts quickly to threats but returns to normal quickly. Specialized noncombat duties during the war. His intellectual qualities are recognized by business, in which he is increasingly successful. Devoted, careful, and intellectual in all his affairs; has a broad grasp of events around him.

CASE 7.—This young man came from a family of very modest means and high standards in the Middle West. He put himself through college on scholarships and by working. His field in physical science was chosen in youth and pursued without question to the present and in a very satisfactory manner. Not athletic, he had troubles in socializing when in school. He is a man of regular habits and devoted to his work, with rather limited interests outside of this work. There have been a few minor psychosomatic episodes connected with interpersonal relations at his job. He has been shrewd in developing his career. He is comfortably married.

CASE 8.—A brilliant young man from the Middle West who is pursuing a scientific career. He has found it difficult to locate himself in a suitable branch of his field of work. More than the usual number of illnesses. A successful family man.

CASE 9.—Tall, impressive, and competent man of science from a home in the Middle West in which some of the patriarchal customs of Europe have been handed down. He has been thorough and systematic in planning the highest grade of training in his field. Has had trouble deciding between basic research and applied science but at length has found a good compromise. Happily married and devoted to both his own and his wife's parents. A quiet leader.

CASE 10.—This young man came from an agricultural community in the Middle West, of upright and strict parents. Since childhood he showed evidence of completing every job with great determination. Has "mental alertness in excess of cultural attainments." Language officer during the war. Steady promotions in his work, which is a field of business. Happily married. Friendly, energetic, dependable.

It happens that each of the five nonsmokers, chosen at random, comes from the Middle-West, whereas all of the heavy smokers are from the Eastern Seaboard. Of the whole group of 51 men who never smoked, 19 came from the Middle West, 26 from the Eastern States, 5 from the West, and 1 from the South. The geographical factor may be partly an artifact because of college admission policies and other factors, and it may contribute a cultural influence towards nonsmoking, such as stricter codes of conduct.

It is evident that the brief case descriptions of the five nonsmokers are in quite marked contrast to those of the five heavy smokers. The nonsmokers are steady, dependable, and hard workers, with stable marriages, histories of specialized, non-

combat war duties, and leading rather quiet progressive lives. The nonsmokers have the more steady dependable sea-going qualities; the smokers are the more dashing, energetic, impulsive, and volatile. A study of values might reveal marked differences. Hypothetically, it may be assumed that an ethical motive of "keeping healthy" would vary in marked degree between the two groups.

Outstanding Traits

In the first years of the Study, while the men were yet in college, the psychiatrists developed, as a first attempt at personality classification, a list of 26 "oustanding traits" which were assigned to persons whenever practicable.[3] Only two of these traits, when compared with smoking habits, showed relationships which reach statistical confidence. The method of comparison is shown in Tables 2 and 3.

Bland affect is defined as "Tendency to be colorless and neutral; plain, undistinguished, uncomplicated and conventional." Inarticulate is defined as "Inability to express oneself in language; inability to describe their feelings and experiences." The nonsmokers have been assigned these two traits in moderate excess.

Although there were only these two outstanding traits which, when compared in smokers and nonsmokers, reached significance, certain of the remaining traits when arranged according to excesses or deficiencies of the nonsmokers (OS) and heavier smokers (HS) groups showed patterns of traits which suggest differences of personality. Thus the five traits which showed the greatest excesses of HS representatives were as follows: least sound ($P=0.25$), less well-integrated ($P=0.06$), lack of purpose and values ($P=0.21$), cultural ($P=0.56$), and practical organizing ($P=0.51$). The five traits which had the greatest deficiencies of HS representatives were as follows: self-conscious and introspective ($P=0.20$), well-integrated ($P=0.13$), inarticulate ($P=0.01$), shy ($P=0.26$), and bland affect ($P=0.01$). The terms used to designate traits are self-explanatory. Terms such as "less well-integrated" and "lack of purpose and values" should not be considered evidence of psychopathology. The subjects were all well within normal psychologic limits of personality, if we define normal broadly as those who function well and usefully, who are healthy and "good" in a number of senses: good fathers, good businessmen, good scientists, teachers, and professional people.

TABLE 2.—*Nonsmokers, Moderate Smokers, and Heavier Smokers Characterized by "Bland Affect"*

Outstanding Trait	Division by Smoking Habits			
	OS, No.	MS, No.	HS, No.	
Bland effect	20	11	13	N=247
Not possessing the trait	40	84	79	$P<0.01$
Totals	60	95	92	

OS=nonsmokers; MS=moderate smokers; HS=heavier smokers. P is determined by the Chi-square test.

TABLE 3.—*Nonsmokers, Moderate Smokers, and Heavier Smokers Characterized by "Inarticulate"*

Outstanding Trait	Division by Smoking Habit			
	OS, No.	MS, No.	HS, No.	
Inarticulate	15	10	9	N=247
Not possessing the trait	45	85	83	P 0.01
Totals	60	95	92	

Among the OS the excesses are of the following traits: well-integrated, inarticulate, bland affect, and physical sciences. The deficiencies are of the following traits: verbal richness, dominance of mood, lack of purpose and values, and less well-integrated. This suggests that the smokers, in contrast to the nonsmokers, may be men who have more difficulties of adjustment but also may be men who are more expressive, perhaps more imaginative, and variable.

For the moderate smokers (MS) group the notable excesses in numbers of men are found for the traits: self-conscious and introspective, shy, vital affect, humanistic, and pragmatic; the notable deficiencies are found for the trait, bland affect (practically alone, with the possible addition of the traits, inarticulate and sensitive affect). All of the five traits having notable excesses of MS have deficiencies of OS and HS. They all

suggest traits not without value for the handling of human situations; that is, if one can imagine one person possessed of all of these outstanding traits (perhaps a confusing concept) one can conceive of the person utilizing one against the other to advantage in dealing with other human beings. There is no case in the series which was assigned all five traits. There is only one assigned four of them (without the trait, vital affect); he is a very successful businessman, classified, however, among the HS! Twenty-one others were assigned three of these outstanding traits apiece. The 21 men distribute themselves as follows: OS 4, MS 11, HS 6.

Certain of the outstanding traits are mutually exclusive, or nearly so, and show contrasting trends which strongly suggest differences in personality among the three types of smokers and nonsmokers. Thus the well-integrated group contains an excess of OS, whereas the less well-integrated group contains an excess of HS. The inarticulate are represented by an excess of OS, whereas a group called "verbal richness" contains a slight excess of MS and HS. The group characterized as shy contains an excess of MS, but that characterized as sociable contains an excess of HS. The group with the trait, bland affect, contains an excess of OS, but that with the trait, vital affect (colorful, rich in expression), an excess of MS. It is interesting that a group of 34 men assigned "unstable autonomic" (periodic anxiety, tremulousness, blushing, perspiring, palpitation, and other functional symptoms) had a normal distribution of smoking habits ($P=0.71$).

A later classification of the men according to "personality stability" based on years of follow-up after college, when compared to the OS, MS, and HS categories, shows an excess of OS and MS for the most stable, and an excess of HS for the least stable ($N=206$, $P=0.09$).

In summary, a study of a comparison between personality traits and smoking habits suggests that nonsmokers, as a group, possess the more stable qualities of dependability and good direction of aims in life, although they are somewhat on the bland, colorless side, whereas the smokers appear to contain more of the men who are energetic, searching for aims and purposes, and perhaps, although less stable, more interesting.

TABLE 4.—*Area of College Major Related to Smoking Habits*

Area of Major	OS, No.	MS, No.	HS, No.	
Natural sciences	31	32	20	
Social studies	17	38	40	N=250
Art, letters, and philosophy	12	25	35	$P<0.01$
Totals	60	95	95	

Choice of Major in College

What a man actually chooses for major in college and what career he goes into are more tangible criteria than are traits which are subjectively assigned. Table 4 shows a relation between general area of major in college and smoking habits.

The OS group shows an excess of men choosing the natural sciences, the MS and HS groups excesses of men choosing social studies and arts or letters. More specifically, the OS group chose particularly chemistry and physics and the HS group, fine arts, foreign languages, and social sciences.

Career Choice

Only one career area reveals a relationship reaching significance. Of the 31 men in science and engineering there is a significant excess of nonsmokers. This is illustrated in Table 5.

Excesses of HS were present for social relations ($P=0.25$), education ($P=0.40$), and writing ($P=0.33$). Excesses of MS

TABLE 5.—*Career Choice Related to Smoking Habits*

Career	OS, No.	MS, No.	HS, No.	
Science	15	11	5	N=247
Nonscience	45	84	87	$P<0.01$
Totals	60	95	92	

were present for business ($P=0.40$) and medicine ($P=0.36$). The distributions of OS, MS, and HS were approximately even for law ($P=0.94$) and for a miscellaneous group of occupations.

In the 1953 questionnaire selected questions taken from the Strong Vocational Interest Blank were inserted. (The Strong Test has been found to be a good predicter of career, particularly for a middle class culture.[4]) Of the 25 career questions compared to smoking habits, 2 showed relationships to the extent that P was less than the 0.01 grade, and for 2 others P was 0.04. The OS group tended to dislike the career of sales manager, the MS group to like it ($P<0.01$). The OS group liked the career of scientific research worker, and the HS group disliked it ($P<0.01$). The careers of farmer and also statistician were liked by MS, disliked by HS ($P=0.04$). Other trends were for OS to like chemistry but to dislike the careers of farmer, corporation lawyer, advertiser, judge, manufacturer, and office manager and for HS to like the career of judge. The trends bear similarity to those for choice of major in college and career.

Armed Services[5]

The tendency in respect to branch of service during World War II was for HS to choose the Army and for OS to choose the Navy or to remain in civilian life (usually in a specialized scientific area allied to the war effort). This tendency is shown in Table 6.

A carefully drawn scale of adjustment to the armed services showed suggestive relationships to smoking habits ($N=214$, $P=0.09$). Excesses of the best adjusted men were among the nonsmokers and moderate smokers. In a small group of 19 men poorly adjusted to the armed services there was a small excess of nonsmokers. Heavier smokers tended to be in excess among the men who had combat duty as opposed to those who had noncombat duty.

TABLE 6.—*Branch of Armed Service Related to Smoking Habits*

Branch of Armed Service	OS, No.	MS, No.	HS, No.	
Army	21	40	57	
Navy (including Coast Guard and Marines)	29	48	26	$N=248$
Civilian	10	7	10	$P<0.01$
Totals	60	95	93	

Psychotype

Somatotonia, cerebrotonia, and viscerotonia, according to the Sheldon Temperament Scale,[6] were compared to smoking habits but revealed very little ($N=243$, $P=0.09$). The OS group was in slight excess among the cerebrotonics (valuing ideas); the MS group, among the somatotonics (liking for action); the HS group, among the viscerotonics (seeking comfort and pleasure). Since smoking, as a so-called "oral pleasure," influenced the assignment of cases to the viscerotonia category, the finding of an excess of HS here can be discounted.

Answering the Questionnaire

The OS group answered the 1955 questionnaire more promptly than did the MS or HS groups ($N=251$, $P=0.03$). Conscientiousness, as a trait of the OS group, goes very well with some of their other traits: bland, well-integrated, sciences. The MS group was intermediate, and the HS group was the slowest in answering. Out of 62 men not answering in four months there were 33 of the HS men. There was no relation between the fullness of the answers in the returned questionnaires and smoking habits.

III. Physiologic and Medical Factors

Physiology, viewed in a broad way, includes personality, for human behavior is the functioning of body in its environment. Even with our relatively crude methods of measuring and recording physiology we should not be surprised to find physiologic differences between smokers and nonsmokers. Out of many comparisons in this area, including some of the medical findings

TABLE 7.—*Respiratory Rate at Time of Medical Examination Related to Smoking Habits*

Respiratory Rate per Minute at Time of Medical Examination	OS, No.	MS, No.	HS, No.	
9–15	29	26	22	
16–19	25	42	41	N=251
20 or more	7	26	33	P <0.01
Totals	61	94	96	

recorded by the physician, are two positive findings which raise important questions: respiratory rate and reflexes.

Respiratory Rate.—Nonsmokers tend to have slower respiratory rates than do smokers. Respiratory rates were taken on two occasions: (1) during the medical examination, with the subject in the sitting position, after the pulse was taken, and (2) at the time of the basal metabolism, the subject having fasted and being recumbent and resting. The latter was recorded by kymograph, allowing for the simultaneous recording of other respiratory functions: tidal air, variation of breathing, number of sighs and swallows. Tables 7 and 8 give the relationships of respiratory rate and the OS, MS, and HS classifications.

Table 7 shows excesses of OS men with slow respiratory rates and excesses of HS men with rapid respiratory rates. (The difference between the respiratory means is greater than 3× P. E. for OS, MS, and for OS, HS differences, but not for MS, HS differences).

Table 8 shows the same trends as Table 7, although P is not as low. Numerous observations have shown that persons have respiratory rates under standard conditions which are characteristically low or high or average. Thus, for our subjects the respiratory rate at the time of the medical examination is rather closely related to the pneumograph respiratory rate, although several weeks or months may have lapsed between the two ($r=0.69$).

TABLE 8.—*Respiratory Rate at Basal State Related to Smoking Habits*

Respiratory Rate per Minute (Pneumograph, Basal State)	OS, No.	MS, No.	HS, No.	
x-10.5	21	18	18	
10.6-14.8	18	35	38	N=194
14.9-x	6	21	19	P 0.05
Totals	45	74	75	

Smoking during college compared with respiratory rates shows similar trends to those given in the tables for respiratory rate during medical examination ($P=0.03$).

Thus there is a tendency for nonsmokers to be slow breathers and for heavier smokers to be rapid breathers. The habit of smoking is, of course, partially a respiratory function. When one is a rapid breather, his tidal air is small and the dead spaces of the lungs are less apt to be filled. There even may be at times an uncomfortable accumulation of carbon dioxide in the blood. Smoking perhaps gratifies these shallower breathers by increasing oxygen intake and blowing off carbon dioxide at times, irrespective of other satisfactions.

If rapid breathers (and, therefore, shallow breathers) tend to smoke more, we should find a relationship between smoking and tidal air, the amount of air inspired with each breath. This is the case when tidal air is compared in the groups OS, MS, and HS, although P (0.13) is not in the range of confidence. Ventilation per minute (the amount of air inhaled every minute) does not show a relationship ($P=0.50$). If anything, the smokers have greater ventilation than the nonsmokers.

Heavier smokers also were found to sigh more frequently during the pneumograph than nonsmokers or moderate smokers ($P=0.22$). Chi-square is not in the range of confidence, but the finding is consistent with the thesis that smokers, as shallower breathers, may need to fill and empty their lungs, at times, more fully.

The heavier smokers swallow more frequently than do the nonsmokers or the moderate smokers ($P=0.04$). Swallows are recorded on the pneumograph as little notches where respiration was suspended while the subject swallowed. Presumably such swallowing is due to excess salivation from the effects of the rubber mouthpiece, psychological or not. The possibility of a

different mouth physiology, that is, a different flow of saliva and more rapid salivary reflex of the smokers, is a point which would be well worth investigating.

Comparisons of OS, MS, and HS with the coefficient of variation of the tidal air ($P=0.20$) and with the standard deviation of the tidal air ($P=0.37$) are not consistent. The tendency is for heavier smokers to have more variability in the depth of respirations.

Respiratory rate and tidal air are also related to outstanding traits. Thus, slow breathers are in excess among the following trait groups: pragmatic, humanistic, cultural, and practical organizing. Rapid breathers are in excess among the following trait groups: shy, ideational, less well-integrated, and asocial. Again we may be dealing with constellations of traits and physiological functions, with smoking habits a part of the constellations. Frequent sighs and swallows also tend to relate themselves to certain traits in the expected fashion.

There seems to be no relationship between respiratory rate and pipe smoking. The above differences occur between nonsmokers and cigarette smokers. Pipe smokers, as a rule, do not inhale, such smoking being more a mouth, lips, and tongue activity than one involving the lungs.

Reflexes

Reflexes were recorded during the physical examination on a threefold scale (normal, increased, decreased) for four locations: knee, ankle, biceps, and abdomen. When a man had active knee jerks he usually had active reflexes elsewhere, but this was not always true. Each of the four reflexes showed similar relationships to smoking habits, but the relationship was most pronounced for the abdominal reflexes (Table 9).*

* The abdominal reflex was obtained by scratching the four quadrants with the sharp point of a wooden applicator. In cases of discrepancies between upper and lower reflexes an average was recorded. Active abdominal reflexes often occurred in the more ticklish subjects. Reflexes which required reinforcement to obtain them were included as "decreased" reflexes.

TABLE 9.—*Abdominal Reflexes as Related to Smoking Habits*

Abdominal Reflexes	OS, No.	MS, No.	HS, No.	
Increased	31	25	21	
Normal	21	46	46	N=224
Decreased	5	14	15	$P<0.01$
Totals	57	85	82	

* The abdominal reflex was obtained by scratching the four quadrants with the sharp point of a wooden applicator. In cases of discrepancies between upper and lower reflexes an average was recorded. Active abdominal reflexes ofter occured in the more ticklish subjects. Reflexes which required reinforcement to obtain them were included as "decreased" reflexes.

The table shows that abdominal reflexes tend to be increased for nonsmokers, decreased or normal for the smokers. The same tendency exists for the biceps jerk ($P=0.05$), ankle jerk ($P=0.07$), and knee jerk ($P=0.19$). It seems unlikely that nicotine ingestion or other effects of smoking could cause reduction of reflexes, which were tested during college when smoking habits had not been fully established.

There are also some interesting differences in outstanding traits for men showing the various types of reflexes. Men with increased reflexes tend to have traits such as well-integrated ($P=0.02$), shy ($P=0.08$), inarticulate ($P=0.10$), bland affect ($P=0.17$), physical science ($P=0.35$), which are mostly those common for nonsmokers. If we group normal and diminished reflexes together, excesses are present for the traits vital affect ($P=0.11$), lack of purpose and values ($P=0.12$), and less well-integrated ($P=0.07$).

There is also a moderate relationship demonstrable between abdominal reflexes and respiratory rates ($P=0.05$), those with increased reflexes tending to be slower breathers, those with normal or decreased reflexes tending to be more rapid breathers. No clear relationship of a like kind was observed, however, between the other reflexes and respiratory rates.

Eating and Drinking Habits

When the participants were in college, a careful dietary history was obtained. Measurements of the servings were made in college dormitories so that the relative

values of food intake for each man could be observed. No definite relationships between smoking habits and daily ingestion of calories ($P=0.68$), fat ($P=0.08$), protein ($P=0.36$), or carbohydrate ($P=0.51$) were found. However, for each of these factors the heavier smokers tended to consume the larger amounts.

The heavier smokers consumed more sugar ($P<0.05$) during college. This is partly associated with a larger coffee indulgence ($P=0.20$). Coffee drinking after college is positively related to smoking ($P<0.01$). Alcohol indulgence is rather closely related, after college, to smoking habits ($P<0.01$). Candy eating was about equal for the three kinds of smokers. Such findings suggest common habit formations which might depend for their nature upon what the environment has to offer.

Weight

One might expect that the heavier smokers would be the ones who would gain more weight because of the evidence of earlier consumption of more calories. Over the years, however, it is the heavier smokers who tended to lose weight. The moderate smokers had among them the most weight gainers, and the nonsmokers had an excess of those whose weight stayed about the same ($P=0.05$). We have no reliable figures for food consumption after college, and so no conclusions as to a relationship between calorie intake and smoking at this time can be made. The gain of weight of men who stop smoking has been studied by Brozek and Keys.[7]

Symptoms During Stress

Although smoking is commonly considered to be a "nervous habit," certain data do not confirm that impression.† During the initial medical examination considerable care was taken to obtain information about the kinds and qualities of symptoms which young men show under stress. (The common stresses experienced were particularly examinations, athletic games, oral recitations, and social occasions, and symptoms were especially anticipatory ones.) The symptoms experienced were the common functional ones of palpitation, easy perspiring, and gastrointestinal or urinary symptoms. Insomnia, headaches, nail biting, frequent colds, hemorrhoids, and other symptoms that might be associated with nervousness under tension were also considered. Note was made of the degree of apprehension which was shown when blood was taken from the arm by venipuncture. The following list is of factors which were compared with the OS, MS and HS categories, with their P values.

	P
Frequency of urination	<0.01
Diarrhea	0.28
Regularity of bowel movement	0.32
Frequency of bowel movement	0.64
Constipation	0.39
Loss of appetite or nausea	0.75
Hemorrhoids	0.70
Gastrointestinal tract irritability	0.24
Circulatory symptoms	0.94
Degree of sinus arrhythmia	0.97
Dermatographia	0.75
Nail biting	0.40
Reaction to venipuncture	0.40
Insomnia	0.60
Frequency of headaches	0.76
History of hay fever or asthma	0.53
Over-all judgment of stability of autonomic nervous system	0.31

Only one of the items showed a relationship which could reasonably occur other than by chance. That item is frequency of urination under stress. The figure of $P<0.01$ is mostly due, however, to only one cell in a 3×3 table. Out of an expected number of 10 nonsmokers only 1 had moderate to marked frequency under stress. The remainder of the table is not suggestive; in fact, the heavier smokers had about the expected distribution among the three factors of frequency of urination which were tested.

† A breakdown of the concept of "nervous" or "nervousness" is needed. Although the degree of symptoms under stress does not here show relations to smoking, it was shown in the previous section that heavy smokers tend to have continuous drive, energy, restlessness, etc., which might be considered a different kind of "nervousness."

Other Medical and Physiologic Factors

Trial comparisons with other factors were run but have not revealed consistent relationships with smoking. Some of these are listed as follows:

	P
Height	0.76
Weight	0.20
Acne vulgaris	0.43
Posture rating	0.45
Number and severity of past illnesses	0.79
Number of medical visits during college	0.20
Frequency of common colds	0.57
Basal metabolism	0.80
Hemoglobin	0.82
Physical fitness: duration of run [8]	0.64
Physical fitness: recovery index [8]	0.20
Physical fitness: lactic acid after run	0.27
Pulse rate (OS, MS, HS)	0.32
Pulse rate (smoking in college)	0.14
Systolic blood pressure	0.84
Diastolic blood pressure	0.98
Number of teeth filled	0.83
Crowding or spacing of teeth	0.40
Occlusion	0.45
Nature of bite	0.72
Handedness	0.96
Blood group	0.69

A rating of the number of skin moles, made during the medical examination, correlated surprisingly with the OS, MS, HS categories ($P=0.01$) (Table 10). An excess of nonsmokers had only rare body moles. Table 10, however, is not consistent, and it is felt that this may be an instance of chance. (Since there is 1 chance in 100 of P equalling 0.01, it is to be expected that chance alone will account for such a relationship when many comparisons are made.) However, it is conceivable that skin moles may be related to tumors of the respiratory epithelium, which is also derived from ectoderm.

TABLE 10.—*Number of Skin Moles Related to Smoking Habits*

Number of Body Moles	OS, No.	MS, No.	HS, No.	
+++ and ++	7	14	8	
+	10	30	43	$N=194$
Rare and 0	26	29	27	$P=0.01$
Totals	43	73	78	

Summary and Conclusions

Two hundred fifty-two selected college men, first studied in 1938 to 1942 and followed by interview and questionnaire since that time, have served as subjects for an investigation of possible differences in personality and physiology between smokers and nonsmokers. The collection of data on smoking was a part of a much larger collection of information from the subjects and was never stressed. After observation of individual smoking habits over the years, a division of men according to smoking habits was as follows: 61 nonsmokers (24.2%), 95 moderate smokers (37.7%), and 96 heavier smokers (38.1%).

A comparison of the case records of the five heaviest smokers (two or more packages of cigarettes daily) with those of five nonsmokers chosen at random showed marked contrasts in personalities. The smokers showed great energy, restlessness, seeking for danger, and a kind of independence which keeps them actively engaged in some enterprise which appeals, and they had difficulties with marriage. The nonsmokers were steady, dependable, and hard workers, with stable marriages and histories of specialized, noncombat war duties, and they led rather quiet progressive lives.

Such differences were statistically borne out for the whole group when comparsions were made with certain personality traits that had already been categorized and with other data which distinguish persons from one another. The nonsmokers, as a group, possess the more stable qualities of dependability and good direction of aims in life, although they are somewhat on the bland uncommunicative side. The group of smokers appears to contain more of the men who are energetic, searching for aims and purposes, verbal, variable, and perhaps, although less stable, more interesting. The nonsmokers tended to major in natural sciences, particularly physics and chemistry, while in college; the smokers tended to choose majors in social studies and arts and letters. The nonsmokers tended to enter

scientific careers; the smokers, careers in social relations, education, and writing. Moderate smokers were represented predominantly in business and medicine. The nonsmokers expressed preference for the career of scientific research worker but dislike for careers such as sales manager, farmer, corporation lawyer. The heavier smokers expressed few preferences but did like the career of judge, disliking that of scientific research worker. In World War II nonsmokers were listed significantly in branches of the Navy, heavier smokers, in the Army. Better adjustment to the armed services was shown by nonsmokers and moderate smokers. Heavier smokers were in excess among the men who had combat duty. There is slight evidence—not conclusive—from a study of psychotype that nonsmokers tend toward cerebrotonia, moderate smokers toward somatotonia, and heavier smokers toward viscerotonia. Nonsmokers answered a questionnaire more promptly than did the others.

The comparison of smoking habits with a wide variety of physiologic and medical data, such as physical fitness, height, weight, number of colds, pulse, blood pressure, and symptoms accompanying stressful experiences, gave largely negative results. Certain findings, however, are noteworthy. There is a strong tendency for nonsmokers to be slow breathers and for heavier smokers to be rapid breathers. This was checked by pneumograph taken at the time of the basal metabolism. Rapid breathing is related to small tidal air and suggests the need for occasional deep breaths (as inhaling cigarettes). Heavier smokers sigh more frequently and swallow more frequently when there is a rubber mouthpiece. Relationships of these respiratory phenomena with personality traits suggest constellations of traits and physiologic factors with smoking habits.

Knee, ankle, biceps, and abdominal reflexes show trends which suggest that they may differentiate smokers as a class from nonsmokers. The reflexes of smokers tend to be reduced. Such reflexes also have suggestive relations with traits and respiratory rates, thus adding to the above-mentioned constellations.

Other findings are that the heavier smokers indulge more in alcohol and in coffee than do the nonsmokers; the moderate smokers tend to gain more weight during the years, the heavier smokers to lose weight; the nonsmokers have few body moles, although this may have been due to chance distribution, despite a value of $P = 0.01$. In this study the attempt was made to cross check whenever possible with similar data (for example, respiratory rate at the time of the medical examination and at the time of basal metabolism, respiratory rates with tidal air, and different kinds of reflexes). This was not possible with skin moles. The characteristics which seem to differentiate smokers from nonsmokers (for simplicity omitting the MS category) may be summarized as in Table 11.

There seems to be little doubt that this group of men can be differentiated as to smoking habits by certain personality traits and physiologic criteria. Although the subjects show a wide geographic and socioeconomic distribution and in many ways have divergent personalities and physiques, they are nevertheless restricted to a segment of college men of a certain age and in some respects to an intellectual class. For

TABLE 11.—*Contrasting Characteristics of Nonsmokers and Heavier Smokers*

Nonsmokers	Heavier Smokers
Bland affect	Cultural
Inarticulate	Lack of purpose and values
Well-integrated	Less well-integrated
Physical sciences	Practical organizing
Most stable personality	Less stable personality
Major in college: natural sciences	Major in college: social studies, arts and letters
Careers: chemistry, physics	Careers: social relations, education
Like: science research worker	Dislike: science research worker
Dislike: sales manager	Like: judge
Psychotype: cerebrotonia	Psychotype: viscerotonia
Answer questionnaires promptly	Delay answering questionnaires
Armed Service: Navy; well-adjusted, noncombat duty	Armed Service: Army; less well-adjusted, combat duty
Respiratory rate: slow	Respiratory rate: rapid
Sighs and swallows: diminished	Sighs and swallows: increased
Reflexes: increased	Reflexes: decreased
Less alcohol and coffee	More alcohol and coffee

this class it seems that, by definition, since the men were selected as "normal," it is "normal" to smoke or not to smoke as the case may be. It is not to be expected that findings could be exactly duplicated in another segment of society. The results, however, suggest strongly that smoking is more than a superficial habit overlayed indiscriminately upon a group of men but has some origins at least in personality and physiologic characteristics. Such characteristics may have just as much right to a place in the etiology of disease as the tobacco smoke ingestion itself. To prove such a point, of course, further investigation is required.

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Sensory Deprivation

Clinical Aspects

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Introduction

Sensory deprivation, a new area of investigation in psychology and psychiatry,[1] has long been familiar to students of certain types of literature. Autobiographical reports from explorers and shipwrecked sailors[2-5] have indicated that gross mental abnormalities such as hallucinations, delusions, and disorientation can occur in persons who are isolated for any length of time. More recently,[6] during the Korean conflict, the effect of sensory deprivation was observed in American prisoners of war who were placed in a highly controlled environment, impoverished of sensory stimuli.

Experimental Studies

The experimental study of sensory deprivation was begun by the psychologists Bexton, Heron, and Scott,[7] in Hebb's Laboratory at McGill University in 1953. These investigators were concerned originally with the practical problem of "lapses in attention" which occur when a man must perform monotonous repetitive acts over a long period of time. In their study, using 22 college men, they placed a subject in a small well-lighted semi-soundproof room for several days. The subject lay on a small cot and wore translucent glasses which transmitted diffuse light but did not permit patterned vision. His movements were partially restricted by encasing his limbs in hollow cylinders. He wore gloves to reduce tactile stimuli. The only sound was a continuous low masking noise. The subject moved about only to eat or go to the toilet. He was given psychological tests before, during, and after the experiment.

In general it was found that the subjects became increasingly irritable and desirous of external stimulation. They did not perform as well on psychological tests as the control group. Many of them reported visual imagery, ranging from extremely simple undifferentiated colors and lines to very complex integrated scenes. A few reported auditory hallucinations and kinesthetic and somaesthetic phenomena. In some, these persisted for hours after they were removed from the situation. This study suggested that adequate sensory stimulation may play a role in maintaining man's normal psychological relationship to his environment.

Lilly,[8] at the National Institute of Mental Health, at Bethesda, attempted to study sensory deprivation by reducing sensory stimulation to a minimum. The subject wore a breathing apparatus and was completely immersed in a tank filled with tepid water;

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thus all stimuli were reduced as much as possible. In the two subjects Lilly studied he found that inner tension or need for stimulation developed within an hour and that visual imagery occurred within two to three hours after immersion. As in the McGill study, the imagery persisted several hours after the subject left the experimental situation.

Bexton et al. and Lilly produced sensory deprivation by reducing the absolute level of stimuli reaching the sensory apparatus. In our work, begun after observing the hallucinatory behavior of poliomyelitis patients in tank-type respirators,[9] we produced sensory deprivation by structuring the sensory environment. This structuring was achieved by making the stimuli montonous and patterned.

Medical, dental, and graduate students and medical house officers, volunteered to spend up to 36 hours in a respirator. The subject was placed in a respirator with the vents open, so that he breathed for himself. He was able to see only a small patch of ceiling. His arms were encased in rigid cylinders, reducing tactile and kinesthetic stimuli to a minimum. The respirator motor was kept running, producing a dull repetitive noise. Bodily needs were met on demand. The subject was not spoken to, but he was told to report whatever occurred to him.

Only 5 of 17 subjects were able to remain in the respirator for the 36 hours.[10] All reported difficulty in concentration, periodic anxiety feelings, and a loss of ability to judge time. Eight of the seventeen reported some distortion of reality, ranging from pseudosomatic delusions to frank visual hallucinations. Four subjects terminated the experiment because of anxiety; two of these in panic tried to release themselves forcibly from the respirator.

The above results indicate that mental abnormalities can be produced in many normal persons when they experience sensory deprivation. If normal persons can develop psychotic-like states under such conditions, how much more likely it is that sick patients, perhaps already perilously near the mental breaking point, can be tipped into psychopathological states by the stress of sensory deprivation. Delirium may be imminent for patients weakened by fever, toxicity, metabolic disturbance, organic brain disease, drug action, or severe emotional strain; sensory deprivation may tip the balance. We have accumulated clinical evidence that sensory deprivation may be one element of importance in the etiology of mental disturbance as a complication of various medical and surgical conditions.

Observations

The following case material has been gathered by us in the course of our regular psychiatric duties at the Boston City Hospital and Massachusetts General Hospital. No attempt has been made to assess the statistical incidence of the types of cases reported here. Cases were sought to illustrate the possible action of sensory deprivation in different kinds of clinical situations. In the case reports which follow, negative findings are omitted for the sake of brevity. In each case the mental abnormalities were reversible.

1. *Poliomyelitis.*—The first patients we saw in whom mental abnormalities developed on the basis of sensory deprivation were patients with bulbar poliomyelitis treated in a tank-type respirator. These patients have been reported elsewhere.[9] Evidence was presented that their psychotic-like states were not due to the poliomyelitis per se or to toxicity, fever, drugs, anoxia, or other metabolic abnormalities but to the conditions of life in a tank-type respirator. Their hallucinations and delusions appeared mainly at night and often involved material concerning traveling in vehicles which resembled the respirator and made similar sounds. The symptoms abated in response to increased sensory stimulation and greater attention from relatives and ward personnel.

2. *Other Neurological Diseases.*—The following cases demonstrate that patients with

other diseases requiring treatment in a tank-type respirator can develop mental abnormalities.

CASE 1.—A 60-year-old man suffered from severe polyneuropathy, with widespread sensory and motor loss and bulbar involvement. In the tank-type respirator he developed "horrible" hallucinations. He saw himself in the basement of a building and thought he was involved in an international intrigue in which he was in imminent danger of being killed. He then imagined himself in a room in Las Vegas with 40 babies. Sometimes at night, either in or out of the respirator, he experienced a peculiar and frightening spatial disorientation in which he could not tell whether he was on his back, stomach, or side. The patient would request that a nurse stand at his right side. He was then able to orient himself.

CASE 2.—A 29-year-old woman had severe post-infectious polyneuritis with marked sensory loss. In the tank-type respirator she became increasingly apprehensive toward evening and developed delusions and hallucinations. She saw her home on fire, heard her children calling and thought them in danger of being burned. Sometimes she saw "bugs on the ceiling." During those periods when she was outside the respirator, her mental state cleared.

In each of these patients the widespread sensory loss due to the polyneuritis may well have contributed to the degree of sensory deprivation already provided by the respirator.[11]

3. *Orthopedic Disorders.*—Patients who require drastic and prolonged immobilization sometimes develop transient psychotic-like states. Treatment in body casts, tongs, or other complex traction is stressful, partly, we believe, because of the sensory deprivation involved. The following case is an example.

CASE 3.—An 85-year-old woman had a fracture of the hip following a fall and was hospitalized for surgical treatment. She had a past history of several other hospital admissions, including two within the previous two years for fractures of the forearm and wrist and of the humerus; she had chronic hypochondriasis and mild depression. On the present admission she became disoriented at night for time and place, attempted to remove herself from traction, and harbored persecutory ideas concerning the ward personnel. It was recommended that she be moved from a private to a semi-private room, that relatives be with her as much as possible, and that a light be left on in her room at night. She responded well and became mentally clear, though still depressed.

It may be thought that the advanced age of this patient played a role in the etiology of the mental disturbance. However, a few months earlier, when hospitalized for a fractured humerus, which did not restrict here severely, she remained mentally clear throughout.

Many orthopedic surgeons are aware of the value of companions, reading, radio, and television in the care of their more restricted patients. The following is an illustrative case.

CASE 4.—A 75-year-old married woman became bedridden following numerous bouts of arthritis and a number of peripheral nerve operations for unexplained and intractable "burning" of the vaginal mucosa. After dark, before her husband returned from work, she sometimes became anxious, confused, disoriented, and vaguely paranoid. The acquisition of a television set which she could operate from her bed resulted in disappearance of these psychotic-like episodes.

4. *Surgery.*—Elderly patients sometimes become mentally decompensated for the first time following an operative procedure.

CASE 5.—A 76-year-old man who had never been in a hospital before was operated on for acute cholecystitis. The only medication given post-operatively was chloral hydrate, 1 gm., for sleep. He did well until three nights later, when he saw some smoke outside his window and developed the idea that the hospital was on fire. He attempted to escape. Restraints made him worse, and he became panic-stricken, confused and disoriented. He thought he was being held in a "Nazi concentration camp."

The patient was moved to a well-lighted room, and someone stayed with him constantly to reassure him. By morning he recovered his mental clarity, and he had no further problem during his hospital period.

5. *Cardiac Disorders.*—The therapy of cardiac disorders often involves prolonged and monotonous immobilization of the patient. Such a regimen, associated with possible cerebral hypoxia and emotional factors related to fear of death, may actively precipitate the mental abnormalities and behavioral changes observed in these patients. That sensory deprivation may play a role is

suggested by the fact that so much of the symptomatology occurs only at night. The following case is an example.

CASE 6.—A 62-year-old widower with hypertensive heart disease was hospitalized with cardiac decompensation. He had been socially and occupationally active up to the day of admission. He was placed on total bed rest in a private room. Two days after admission he began to get out of bed and wander dazedly through the corridors. Restraints upset him badly. Even after complete digitalization and return of cardiac compensation, the behavior abnormalities continued. They rapidly disappeared when he was transferred from a single room into a ward, which provided a more stimulating environment.

6. *Blindness.*—Ophthalmologic surgeons have recognized the connection between loss of vision and mental abnormality.[12-14] In 1928 Greenwood [15] suggested that in postoperative cataract patients who developed mental abnormalities the eye patch should be removed from the unoperated-on eye in order to increase the patient's contact with his environment. Linn et al., in 1953,[17] followed this suggestion in connection with their own study of this problem and found that 6 of 13 patients showed marked improvement immediately and 3 others improved within 48 hours.

In the following case, both emotional factors and sensory deprivation probably played a part.

CASE 7.—A 71-year-old woman with a previous history of nervous disorder and depression developed complete blindness as the result of unrecognized acute glaucoma. Hospitalization, medical treatments, and iridectomies failed to restore her vision. Back at home, she could not adjust to her handicap and became increasingly tense and agitated. Two months after the onset of the blindness she broke down in prolonged crying, sank into a stuperous state, and emerged with striking and frightening visual hallucinations. She saw little children playing around her, but they vanished when she reached out to touch them. She seemed to be out of her home surrounded by crowds of people so that she could hardly move.

Admitted to the hospital, she was a problem in management because of her confused restlessness and inability to cooperate. She became disoriented in time and place and continued to hallucinate actively. Chlorpromazine and chloral hydrate were administered, with only slight improvement. She was then given a great deal of attention from family, hospital personnel, and consultants, whereupon her psychotic symptoms disappeared in the course of the next three days. She has since remained somewhat depressed but has been free of other mental disturbance.

A study of the congenitally blind which we now have in progress [18] may help elucidate the effects of sensory deprivation on normal psychological processes.

7. *Deafness.*—Loss of hearing may also be accompanied by mental abnormality.[19-22] We have made a psychiatric study of students with congenital and acquired deafness [23] and have obtained data in the realm of dream and fantasy life. There appears to be a relationship between both the vividly colored three-dimentional dreams and the conscious fantasies of some of the congenitally deaf to the projections of visual imagery experienced by both patients and experimental subjects exposed to sensory deprivation. In addition, some students with acquired deafness exhibit marked difficulty in judging temporal sequences, though their time orientation is intact.

8. *Public Health.*—The effects of sensory deprivation have relevance to the increasing accident rate on the highways. A recent study [24] revealed that 30 of 50 long-distance truck drivers had visual hallucinations. The monotony of driving on the new superhighways is conducive to personal error, perhaps in part because of the phenomena associated with the lack of stimulation or sensory deprivation. It is not only falling asleep which is the grave danger but inattention, mild disorientation, and excessive fantasy formation, phenomena characteristic of those subjected to sensory deprivation.

The following case demonstrates some of the relationship between driving fatigue, sleeplessness, and pseudohallucinations.

CASE 8.—An 18-year-old college student took a long automobile trip directly after midyear examinations. He drove alone several hundred miles without a radio, slept little for two nights, partly because of engine trouble and a snowstorm, and finally went to bed on the morning of the third day before he had seen anyone to talk with. Looking up at the ceiling he suddenly had vivid hallu-

Summary of Mental Abnormalities

Case	Age, Yr.	Manifest Anxiety	Disorientation			Delusions	Hallucinations		
			Person	Place	Time		Visual	Auditory	Somesthetic
Poliomyelitis, composite of 9 cases [1]	11–43	x	x	x	x	x	x	x	x
Other neurological diseases									
1. Polyneuropathy	60	x		x		x	x		
2. Polyneuropathy	29	x		x		x	x	x	
Orthopedic disorders									
3. Fractured hip	85	x		x	x	x			
4. Arthritis	75	x			x	x			
Surgery									
5. Cholecystectomy	76	x		x	x	x			
Cardiac disorders									
6. Congestive failure	62	x		x	x				
Blindness									
7. Acute glaucoma	71	x		x	x	x	x		
Deafness, composite of 17 cases [2]	19–23	x			?				
Public health									
8. Car driving fatigue	18						x		

cinations in bright colors. He saw buildings and many groups of people similar to those he had seen on his trip. He thought he could see over the top of one of the buildings. Two doors in the building opened and closed periodically as people walked in and out. He felt as though he were "two feet off the ground, floating in air, observing things." It was totally unlike a dream. He was interested in what he saw and tried to keep the experience going. At one point when it faded he was able to bring it back.

On other occasions this student experienced brief lapses in awareness while driving, particularly on turnpikes. He sometimes fancied that he saw people on the road and slowed down unnecessarily. Listening to the radio prevented this phenomenon.

A tabular summary of the abnormal mental findings in the above eight cases and in the poliomyelitis patients and deaf students may be seen by referring to the accompanying Table.

Comment

The systematic investigation of sensory deprivation has heretofore been limited to the field of experimental psychiatry. In the course of our work it became apparent that this concept might have implications for general medicine. We have collected a group of medical and surgical patients in whom transient psychotic-like symptoms appear. The symptoms are found in both the old and the young, in both men and women, and in diverse acute and chronic clinical conditions. The symptoms seem to be unrelated to the medical disease but do appear to be related to the adequacy of the patient's sensory environment. The onset occurs either shortly after the sensory environment becomes impoverished, usually within 12 to 72 hours, or as long after as 2 months. This psychotic-like state does not respond to the usual medical or psychiatric regimen, but it characteristically does respond promptly to appropriate manipulation of the sensory environment. The symptoms of the psychotic-like state include pathological manifest anxiety, delusions, and visual and, occasionally, auditory and somesthetic hallucinations. These symptoms appear to be conditioned by the premorbid personality of the individual person. The clinical state we are describing most clearly resembles the so-called "toxic delirious states," from which it is to be differentiated by both the absence of toxicity and the prompt response to manipulation of the sensory environment.

Since we are introducing a new concept in clinical medicine, it is essential that we examine in some detail the alternative explanations. The question may be asked, were the patients presented suffering from toxic or metabolic psychosis? In most cases of toxic or metabolic delirium there are signs of infection, neoplasm, drug ingestion, hypoxia, or other metabolic disturbance. There is no evidence that these factors were present in our patients while the mental symptoms were occuring. We recognize, however, that in seriously ill patients, though laboratory findings may be normal, some degree of abnormal metabolism may be present. Such metabolic factors alone can-

not explain the behavior of our patients, and it is reasonable to assume that sensory deprivation must have played a part in the psychotic process, since therapy was effected by manipulation of the sensory environment.

Some of our patients were given medication during the course of their therapy. Certain drugs, particularly hypnotics, which reduce the levels of awareness and impair sensory discrimination, can act synergistically with an impoverished sensory environment to produce and potentiate mental abnormalities. However, neither the administration or withdrawal of medication appeared to coincide with the mental symptomatology we observed.

Any disease associated with hypoxia may produce alterations of oxygen-dependent central nervous system metabolism. There is no evidence, however, to support the contention that hypoxia alone can account for mental abnormalities comparable to those observed in our cases.

Six of our eight cases were 60 years of age or older. It is possible that these elderly patients had varying degrees of organic brain disease. It is true that the acute and rapidly reversible mental changes seen in our elderly patients may be considered to be typical of early organic brain disease. It should be noted, however, that once the symptoms disappeared as the result of sensory stimulation, they did not recur.

In the aged there is a decrease in the efficiency of the sensory apparatus [25-27] and a lessened capacity to adapt to surroundings which present the patient with new and unfamiliar sensory stimuli. This form of sensory deprivation may result in the phenomenon of senile nocturnal delirium.[28]

Could the mental abnormalities in our patients have been produced by emotional reactions? Each of these patients, with the exception of the student (Case 8), had a severe disabling disease. It might be postulated that the delusions and hallucinations are a reaction to the overwhelming threat of suffering, invalidism, and death.[29,30] The psychological mechanism of denial, as suggested by Linn [16,17] in his observations of postoperative cataract patients, may serve as a defense against such a threat. Psychological phenomena undoubtedly operate in our patients too, but in order to make understandable the rapid reversibility resulting from sensory stimulation, it is necessary to add the additional etiological element of sensory deprivation.

Were our patients prepsychotic? Was it the stress of the acute illness rather than sensory deprivation that brought on the mental abnormalities? One patient did have a past history of depression and hypochondriasis. None of the patients had a past history of psychosis or grossly abnormal behavioral tendencies. Furthermore, psychotic symptomatology did not recur once sensory deprivation was ended.

Social isolation is one aspect of sensory deprivation. Most of our patients developed psychotic symptoms when they were alone. However, the presence of another person was not always sufficient to alleviate the symptoms. Additional sensory stimuli were necessary before the psychotic process could be reversed.

We do not believe that our patients merely were experiencing hypnagogic phenomena. Most of them were wide-awake, had their eyes open, and were able to recognize and converse with hospital personnel when reporting their experiences.

Having considered organic and emotional factors which may play a role in delirious states, we believe we have established that sensory deprivation is one other element that must be thought of. For example, if an elderly patient becomes disturbed and noisy, he may be given sedative drugs and be moved to an isolated room because of consideration for other patients. Such a maneuver, unfortunately, might enhance sensory deprivation and actually heighten the aberrant behavior. Thus, failure to consider the presence of sensory deprivation

may lead to therapy which is diametrically opposed to that which is appropriate.

The therapy of sensory deprivation is actually its prevention. It may be as simple a matter as the avoidance of darkness, silence, and solitude. It may involve keeping on a night light, the provision of a radio or television set, or the presence of another person. In more complex form it would include ward organization to enhance social contact, increased attention to more stimulating hospital decor, and music, occupational, and recreational therapy. At the same time, it becomes obvious that unduly prolonged bed rest, the use of "back wards" in psychiatric and general hospitals, and the excessive use of sedatives or restraints should be avoided.

The theoretical basis for sensory deprivation is as yet incompletely understood, though certain neurophysiological and psychoanalytic concepts have an obvious relevance.[31-35] These will not be discussed here.

Sensory deprivation plays a part in certain current problems of public health. The monotony of the new superhighway reduces sensory stimulation and may thereby jeopardize the normal psychological functioning of the driver. The monotony of the repetitive task in assembly-line work may act similarly. Sensory deprivation thus plays a role in automobile and industrial accidents. The military applicability of the concept obviously applies to the lonely soldier at his post, the radar observer at his scope, and the pilot of the future in his interplanetary ship.

As we stated before, we are presenting the cases in this paper in order to illustrate the applicability of the concept of sensory deprivation in clinical medicine. We are not attempting to establish its validity on a statistical basis. The establishment of its validity can be accomplished only by the work of many investigators who will compile and examine their own clinical material with this concept in mind.

Summary

Sensory deprivation is a new area of investigation in psychology and psychiatry. It has been familiar in literature through the autobiographical reports of explorers and shipwrecked sailors and, more recently, in the accounts of American prisoners-of-war. The experimental approach to the subject dates from the present decade.

We have attempted to extend our own investigative work by seeking practical applications in clinical medicine. We report a number of patients in whom mental abnormality occurred during the course of severe illness. In each of these patients, we believe that sensory deprivation, largely in the form of darkness, silence, and solitude, was responsible for the mental abnormality. We were able to demonstrate that sensory stimulation could relieve the mental symptoms. These observations are discussed, and implications are drawn for the fields of general medicine and public health.

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The Sensitivity of Staphylococci to Antibiotics

A Five-Year Study of Sensitivity, Usage, and Cross Resistance in a General Hospital

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The purpose of this report is to present data regarding the sensitivities and cross resistances of staphylococci to antibiotics in a manner that will be of maximum use and interest. In addition, certain fundamental conclusions regarding the influence of chemotherapy on the resistance of bacteria that are suggested by these data will be considered.

The current problem of infections due to staphylococci has been the subject of numerous publications, and several authors have commented upon the need for precise long-term epidemiological studies of staphylococci which might help delineate mechanisms of changing resistances to antibiotics and also which might point the way to logical solutions of this problem.[1-6] The present study was begun in 1952 at the Milwaukee County General Hospital, and since that time 500 patients with staphylococci infections have been studied at the bedside and through in vitro tests of the organisms isolated from them. The clinical pictures of staphylococcic infections, correlations between sensitivity tests and clinical results, and studies of the pathogenesis of staphylococci infections will be the subjects of later reports. The data regarding the sensitivity of the staphylococci to the antibiotics are being presented first because they have been of definite aid to us in our approach to patients with severe staphylococci infections, and it is hoped they will be of similiar aid to physicians confronted with patients who must be treated before the results of precise laboratory studies are available. Data regarding possible mechanisms of changing resistance to antibiotics and correlations between usage and resistance changes are being presented as important by-products of this study. The extensive literature upon this subject will not be reviewed but will be commented upon where it is relevant to our work.

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I. The Relative Sensitivities of Staphylococci to the Various Antibiotics

Materials and Methods

Five hundred strains of pathogenic coagulase-positive staphylococci isolated from patients at the Milwaukee County General Hospital between 1952 and 1956 were tested for sensitivity to the antibiotics by the tube-dilution method.[7] In this method, 0.5 ml. of a 1:100 dilution in dextrose broth of an 18-hour growth of bacteria is added to tubes containing decreasing concentrations of antibiotics dissolved in distilled water. The bacteriostatic end-point (which was used throughout this study) is read as the lowest concentration of antibiotic that prevents visible growth of bacteria at the end of 18 hours' incubation at 37 C.

Results

The relative activity of antibiotics against the entire group of strains is shown on Table 1. It should be pointed out that chloramphenicol and nitrofurantoin are given a rather marked advantage in regard to the inhibitory concentration at which bacteria are considered to be sensitive (25μg. per milliliter is used as a critical level for these agents, but 3μg. per milliliter is used for the rest of the antimicrobial agents), but we

TABLE 1.—*The Relative Activity of the Antibiotics Against Strains of Coagulase-Positive Staphylococci Isolated from Patients at the Milwaukee County General Hospital Between Jan. 1, 1952, and Dec. 31, 1956*

Antibiotic	Strains Tested, No.	Amount of Antibiotic, μg./Ml.	% Sensitive	Clinical Rank
Neomycin	500	3	96.6	1
Nitrofurantoin *	300	25	95	2
Vancomycin	74	3	89	3
Chloramphenicol	500	25	88	4
Novobiocin	100	3	82	5
Erythromycin	500	3	77	6
Chlortetracycline	500	3	48.8	7
Tetracycline	300	3	48.3	8
Oxytetracycline	500	3	43.8	9
Streptomycin	500	3	34.8	10
Oleandomycin	45	3	31	11
Bacitracin	300	3	27	12
Penicillin	500	3	25.4	13

* This is not an antibiotic.

have repeatedly observed clinical responses in infections treated with chloramphenicol and nitrofurantoin when the organisms need 25μg. per milliliter for inhibition, whereas other agents have rarely been effective unless the bacteria are inhibited by 3μg. per milliliter or less. Table 1 emphasizes the fact that neomycin remains the most potent antibiotic against staphylococci, and this fact plus the rapid bactericidal action of neomycin is the reason why we have repeatedly recommended the use of intravenous neomycin in overwhelming staphylococcal endocarditis and bacteremia.[8] The toxicity of neomycin, however, precludes its use routinely for staphylococcal infections.[8] Thus, the current drugs of choice should be vancomycin, chloramphenicol, novobiocin and erythromycin (Table 1). Nitrofurantoin is effective only in urinary tract infections but should be used first in those genitourinary infections caused by staphylococci (Table 1).

II. Cross Resistances and Sensitivities of Staphylococci to the Various Antimicrobial Agents

Materials and Methods

Cross resistances and sensitivities were studied by finding what percentages of strains resistant to each of the antbiotics also were resistant to each of the other antibiotics (Table 2). Arbitrary clinical levels of sensitivity (see above) were used because of our desire to present the data in the manner calculated to be of maximum clinical use. An additional use of the cross resistant data was to construct a "what to change to" table (Table 3), in which the antibiotics that have the best chance of giving a response after the failure of each other antibiotic are listed. This was done by noting the sensitivities of the strains resistant to each of the antibiotics and selecting for each antibiotic those agents that were active against the greatest number of strains that were resistant to it.

The cross resistance data also were used to determine the combinations of antibiotics that would

TABLE 2.—*The Per Cent of Each Group of Strains Resistant to Each Antibiotic That Are Also Resistant to Each of the Other Antibiotics* *

Antibiotic	A	C	T	S	P	N	B	E	Ac	F	Al	O	V
Chlortetracycline	100 (315)	17	88	91	91	6	83	37	84	3	40	91	18
Chloramphenicol	83	100 (64)	77	72	90	17	90	63	88	5	50	100	5
Oxytetracycline	83	18	100 (328)	92	89	6	85	34	79	3	33	84	13
Streptomycin	71	14	76	100 (399)	90	6	76	33	66	4	31	73	14
Penicillin	64	13	66	81	100 (437)	5	81	30	60	3	32	71	14
Neomycin	70	33	74	89	81	100 (27)	85	56	81	30	93	100	0
Bacitracin	54	14	58	71	83	1	100 (315)	33	39	3	27	50	8
Erythromycin	74	26	72	86	87	10	73	100 (154)	92	8	64	100	32
Tetracycline	83	21	83	83	84	9	66	53	100 (192)	5	35	41	9
Nitrofurantoin	20	8	20	40	32	20	20	28	7	100 (15)	40	4	3
Novobiocin	65	16	57	65	73	14	76	51	57	5	100 (37)	59	10
Oleandomycin	58	27	57	61	65	6	84	47	75	6	24	100 (31)	6
Vancomycin	57	57	43	57	64	0	100	50	86	21	57	21	100 (12)

* Numbers in the horizontal lines represent the percentage of strains resistant to antibiotics at the left that are also resistant to the antibiotic under which they appear. A indicates chlortetracycline; C, chloramphenicol; T, oxytetracycline; S, streptomycin; P, penicillin; N, neomycin; B, bacitracin; E, erythromycin; Ac, tetracycline; F, nitrofurantoin; Al, novobiocin; O, oleandomycin; V, vancomycin. Parenthetical figures are number of resistant strains called 100%.

TABLE 3.—*Order of the Antibiotics to Which There Most Likely Will Be a Response in Patients Who Are Not Responding to a Particular Antibiotic, i. e., "A What to Change to Chart" Derived from Table 2*

Antibiotic to Which There Is No Response	Antibiotics to Which There Is Most Likely to Be Response (in Order)
Chlortetracycline	Nitrofurantoin, neomycin, chloramphenicol
Chloramphenicol	Vancomycin, nitrofurantoin, neomycin
Oxytetracycline	Nitrofurantoin, neomycin, vancomycin
Streptomycin	Nitrofurantoin, neomycin, chloramphenicol, vancomycin
Penicillin	Nitrofurantoin, neomycin, chloramphenicol, vancomycin
Neomycin	Vancomycin, nitrofurantoin, chloramphenicol
Bacitracin	Neomycin, nitrofurantoin, vancomycin
Erythromycin	Nitrofurantoin, neomycin, chloramphenicol
Tetracycline	Nitrofurantoin, vancomycin, neomycin
Nitrofurantoin	Vancomycin, oleandomycin, tetracycline, chloramphenicol
Novobiocin	Nitrofurantoin, vancomycin, neomycin, chloramphenicol
Oleandomycin	Vancomycin, nitrofurantoin, neomycin, novobiocin
Vancomycin	Neomycin, nitrofurantoin, oleandomycin

have the best chance of inhibiting a strain of Staphylococcus for which no sensitivity data were available. These theoretical optimal combinations were derived by adding to a first antibiotic that antibiotic which was most effective against strains resistant to that first antibiotic (Table 4). Tables 3 and 4 are essentially restatements of Table 2 that are presented to give these data optimal practical use.

Results

The percentage of strains that were resistant to each antibiotic that were also resistant to each of the other antibiotics are shown on Table 2. This Table has been of great use when patients were encountered with staphylococcic infections that were not responding to the antibiotic being used. In this situation the antibiotic most likely to be effective can be determined by reading in Table 2 the horizontal line after the antibiotic to which there has been no response. The next indicated antibiotic is the antibiotic to which are sensitive the greatest number of strains resistant to the first agent. Table 3 represents the tabulation that was made in the above manner from Table 2.

TABLE 4.—*The Combinations Involving the Most Effective Antibiotics Plus Another Agent That Gives the Best Theoretical Action Against Coagulase-Positive Staphylococci*

Combination	Sensitive To First-Named Agent, %	Coagulase-Positive Staph. Strains Theoretically Sensitive to Combination, %
Neomycin & vancomycin	96.6	100
Neomycin & nitrofurantoin	96.6	100
Nitrofurantoin & vancomycin	95	99
Nitrofurantoin & oleandomycin	95	99
Vancomycin & oleandomycin	89	98
Chloramphenicol & nitrofurantoin	88	99
Chloramphenicol & vancomycin	88	99
Novobiocin & nitrofurantoin	82	99
Novobiocin & vancomycin	82	98
Erythromycin & nitrofurantoin	77	98
Erythromycin & neomycin	77	98
Chloramphenicol & erythromycin	88	96
Vancomycin & erythromycin	89	95
Tetracycline & oleandomycin	48	69
Streptomycin & penicillin	35	42

Table 4 shows the rank of theoretical combinations against staphylococci and the percentage of strains that should be sensitive to one or the other antibiotic in a given combination. The fact that there are 13 combinations of agents that are effective against 95% of strains of staphylococci is worthy of emphasis. The low comparative rank of some combinations that have had particular exploitation also is worthy of note.[10,11]

III. Significant Changes in the Sensitivity to the Antibiotics of Staphylococci That Have Occurred Between 1952 and 1956 and Correlations Between These Changes and Usages

Materials and Methods

In 1952, at the inception of this study, it was decided to test a regular number of strains of staphylococci each month and in this way to keep track of changes in sensitivity to the various agents as they occurred. An advantage of the tube-dilution method for determining bacterial sensitivity to antibiotics is that the results lend themselves to a statistical analysis in regard to changes in the sensitivity of different species to the antibiotics.[12] One hundred strains of staphylococci were tested each year, and changes in their sensitivity to any of the antibiotics were determined by analyzing the differences in the means of the numbers of the dilutions necessary for inhibition.[12,13] A significant change in sensitivity was accepted when the differences between these means gave a P value of less that 0.01 (i. e., there was less than 1 chance in 100 that the differences in means that were found could have resulted from chance alone). Changes in antibiotic sensitivity that occurred yearly between 1952 and 1956 were determined.

Results

Significant changes that occurred in the sensitivity to antibiotics of strains of staphylococci isolated each year from 1952 through 1956 and changes that occurred when the 1952 strains were compared with those isolated in 1956 are shown on Table 5. Table 5 and Table 6 tend to emphasize the fact that there have been relatively few significant changes in the resistance of staphylococci to the antibiotics. In the five-year period, there were only five instances in which a yearly increase in resistance to an antibiotic was noted and three cases in which staphylococci became more sensitive to an agent. It is worthy of comment that two of the five increases in resistance involved erythromycin, which others have found to be particularly prone to be associated with increases in resistance, and that two of the changes were involved with neomycin and bacitracin, which were not used in great quantities at our hospital.[14,15] When the 1952 sensitivities were compared to the 1956 sensitivities, the only antibiotic to which a significant change was noted was erythromycin. When one considers that if each of six antibiotics that were followed had changed yearly 45 instances of change would have been recorded, it is apparent that all in all, the staphylococci remained relatively stable in regard to their sensitivities to the antibiotics.

TABLE 5.—*Comparison of Antibiotic Usage and Resistances of Coagulase-Positive Staphylococci*

Antibiotic	Year	Total Hospital Usage, Kg.	Resistance *
Chlortetracycline	1952	†	5.32
	1953	4.784	6.20
	1954	2.5724	6.52
	1955	1.067	7.00 ‡
	1956	0.771	5.78
Chloramphenicol	1952	†	7.10
	1953	1.2755	6.87
	1954	1.6525	6.60
	1955	6.7675	6.89 ‡
	1956	16.710	7.45
Oxytetracycline	1952	†	6.58
	1953	3.002	7.00
	1954	1.9155	6.12
	1955	0.843	5.90
	1956	1.368	6.21
Streptomycin	1952	†	8.19
	1953	1.850	8.40 ‡
	1954	2.128	6.52
	1955	0.6855	6.73
	1956	0.4575	7.79
Penicillin	1952	†	8.20
	1953	14.9708	8.51
	1954	10.2935	7.80
	1955	13.6034	7.01
	1956	15.1674	7.58
Neomycin	1952	†	1.91
	1953	0.514	2.38
	1954	0.7135	2.04
	1955	1.146	1.76 ‡
	1956	4.084	2.33
Erythromycin	1952	†	3.91 ‡
	1953	0.913	4.49
	1954	1.9728	5.19
	1955	2.674	4.84 ‡
	1956	8.988	6.49
Bacitracin	1952	†	†
	1953	0.052	†
	1954	0.0084	5.80
	1955	0.089	6.27 ‡
	1956	0.040	8.05
Tetracycline §	1954	1.1631	5.80
	1955	2.032	5.58
	1956	5.002	6.00
Nitrofurantoin §	1954	0.1665	6.48 ‡
	1955	0.409	5.99
	1956	0.095	6.28
All antibiotics	1952	†	5.89
	1953	27.3613	6.26
	1954	22.5862	5.89
	1955	29.3164	5.80
	1956	52.6829	6.07
		131.9468 ‖	5.87

* Mean of number of tube dilutions necessary for inhibition.
† No data available.
‡ Significant changes, i. e., P value <0.01. The formula used to determine significance is as follows [12, 13]:

$$T = \frac{D}{\sqrt{\frac{S_1^2}{100} + \frac{S_2^2}{100}}}$$

D indicates difference of means; S_1, standard deviation of means of 1st sample; S_2, standard deviation of means of 2d sample.

‖ Total use in Milwaukee County General Hospital of the antibiotics on the chart—not necessarily used because of a coagulase-positive staphylococci infection.
§ Tetracycline and nitrofurantoin were not in use before 1954.

TABLE 6.—*Significant and Insignificant Changes That Occurred in the Sensitivity of Staphylococci to Antibiotics Between 1952 and 1956 as Compared to Changes in Antibiotic Usage During This Period*

Antibiotic	Mean of Tube Dilution Necessary for Inhibition		Significance of Change	Change in Usage, %
	1st Year	2d Year		
The tetracyclines 1954–1955	6.1	6.2	None	—69
Chloramphenicol 1954–1955	6.6	6.9	None	+410
Penicillin 1953–1954	8.5	7.8	None	—69
Chloramphenicol 1955–1956	6.9	7.5	More resistant	+247
Erythromycin 1952–1953	3.9	4.5	More resistant	No data
Erythromycin 1955–1956	4.8	6.5	More resistant	+335
Bacitracin 1955–1956	6.3	8	More resistant	—45
Neomycin 1955–1956	1.8	2.3	More resistant	+355
Chlortetracycline 1955–1956	7	5.8	More sensitive	—72 (All tetracyclines)
Streptomycin 1953–1954	8.4	6.5	More sensitive	+115
Nitrofurantoin 1954–1955	6.5	5.9	More sensitive	—232

IV. Studies to Help Delineate Reasons for Changes in the Staphylococcic Sensitivities to Antibiotics and Possible Variations Within Certain Groups of Strains

A. Influence of Usage of the Antibiotics on Changes in the Sensitivities of Staphylococci to Them

Materials and Methods

The daily ward requisitions for antibiotics were examined for each ward from the period 1954 through 1956. In this way the amount of each antibiotic that was used in the hospital during each month of these years was determined. In every instance where an increase or decrease in antibiotic sensitivity was found (see above) the amount of increase or decrease in the usage of that agent during the year preceding and during the change was noted. For comparison, changes in cases where no increase or decrease in resistance to the antibiotic occurred also were noted.

Results

It was impossible to analyze in a statistical manner whether or not there was a direct relationship between usage and increase in antibiotic resistance because there is no way of determining what constitutes a significant increase in usage. However, a perusal of Tables 5, 6, and 7 will show that changes in usage were approximately the same in cases in which no change in sensitivity was noted as in those in which significant changes occurred.

In Table 5, where the total antibiotic usage was compared with total average resistance to all of the antibiotics, it is particularly striking that when total usage almost doubled in 1956 the staphylococci as a group were no more resistant to antibiotics than they were in 1953.

In Table 7 an attempt was made to compare usage to changes in sensitivity by correlating, irrespective of degree, increases or decreases in usage with increases or decreases in resistance. In the 15 instances when yearly usage increased, resistance to the antibiotics concerned increased in 9 instances. In 12 instances when usage decreased, sensitivity increased 8 times. Thus, a positive correlation between usage and resistance occurred 17 out of 27 times that it could be compared (Table 7). When significant changes only were considered, a positive correlation was found in five of the seven cases in which data were available (Table 7).

Our interpretation of these data is that they do not suggest a strong relationship between usage of antibiotics and the resistance of staphylococci to them. On the other hand, they do not conclusively prove that usage may not be concerned in changes in resistance of staphylococci to antibiotics.

TABLE 7.—*Effect of Usage of Antibiotics on Sensitivity of Coagulase-Positive Staphylococci to Them: Data Obtained by Comparing Means for Each Antibiotic for Each Year with Use of Each Antibiotic Each Year*

Direction of Antibiotic Usage	Times Means Increased (More Resistant), No. *	Times Means Decreased (More Sensitive), No. *
Up	9	6
Down	4	8
Cases Where There Was a Significant Change		
Up	3	2/5
Down	½	2/5

* Means greater or less, not necessarily significantly. For significant changes see Tables 5 and 6. No usage data for erythromycin from 1952 to 1953.

B. Differences in the Sensitivity to Antibiotics of Strains of Staphylococci Isolated from Different Sources and at Different Times of the Year

Materials and Methods

Groups of staphylococcic strains were compared in respect to their sensitivity to antibiotics by statistical analysis of the differences of the means of the dilutions of antibiotics necessary for inhibition of each group[12,18] (Table 5). Groups of staphylococci isolated from the blood, from wounds, from the stool, from members of the house staff, from individual wards, and from a near-by private hospital were compared. The strains in each of the groups were selected carefully to insure that the only variables were the factors under consideration. For instance, when two wards were compared, an equal number of strains from wounds and from blood were selected from each ward. Similarly, when strains from blood and from wounds were compared, an equal distribution from wards was selected. The sensitivities to all the antibiotics were compared in each case, i.e., when strains from blood and wounds were compared, their sensitivities to 10 antibiotics were analyzed statistically. For ease of presentation only cases in

TABLE 8.—*Significant Differences in Sensitivity to Antibiotics of Coagulase-Positive Staphylococci Isolated from Different Sources, Different Hospitals, and at Different Times of the Year*

Type of Grouping	Strains, No.	Significant Differences
Blood	60	Blood isolates more sensitive to penicillin; wound isolates more sensitive to bacitracin
Wounds	60	
Wounds	70	Stool isolates more resistant to all tetracyclines, chloramphenicol, & erythromycin
Stool	13	
Stool	13	Stool isolates more resistant to the 3 tetracyclines, streptomycin, penicillin, & erythromycin
Blood	43	
Warmest month	44	Cold-weather strains more sensitive to chloramphenicol
Coldest month	54	
St. Joseph's Hospital	288	Strains from St. Joseph's Hospital more sensitive to chloramphenicol, penicillin, & erythromycin
Milwaukee County General Hospital 1955 and 1956	200	
House staff (phage-typed)	12	House-staff strains more sensitive to penicillin & erythromycin
Patients (phage-typed)	17	
Ward 2W (all sources)	26	No significant differences in sensitivity between wards
Ward 3E (all sources)	26	

which significant differences were found will be mentioned and tabulated.

Strains during the hottest month of each year also were compared with those isolated during the coldest months of the same year. These studies were undertaken to determine if highly individual groups of strains predominated in certain niches or under certain circumstances.

Results

The results of the comparisons of seven groups of strains are summarized in Table 8. The findings of note and their possible interpretations are as follows.

(*a*) The group of strains isolated from stools were markedly more resistant to antibiotics than those found in the blood stream or in wounds (Table 8). This is probably because they were all isolated from patients who had staphylococcic diarrheas subsequent to antibiotic therapy. This should emphasize the fact that while on an over-all population basis the antibiotics might not have a great influence, in the "small worlds" of the persons who are receiving antibiotics and who develop superinfections the selective influence of antibiotics may be profound and tend to result in the emergence of relatively antibiotic-resistant organisms.

(*b*) There was relatively little difference in antibiotic sensitivity between staphylococci isolated from blood and from wounds. The fact that staphylococci from the blood stream were more sensitive to penicillin than those isolated from wounds may be due to the fact that many of bacteremias began outside the hospital where it has been reported staphylococci are usually more penicillin-sensitive.[16,17]

(*c*) There were no significant differences in antibiotic sensitivities between staphylococci isolated from a surgical ward (3E) and those isolated from a medical ward (2W) when groups of strains were selected from the same sources. This was of particular interest when antibiotic usages on the two wards were compared (Table 9). It can be noted that while the total usage in grams was quite comparable, considerably more of the tetracyclines and streptomycin were used on the surgical ward. This did not apparently cause the staphylococci isolated from that ward to become more resistant than those isolated from other places within the hospital.

(*d*) The noses and throats of 87 members of the house staff of the hospital were cultured, and coagulase-positive staphylococci were isolated from 23 persons. Repeat cultures done on all of these people were again positive one month later. Our carrier rate of 27% thus compared closely to that of others.[18] Phage typing was done on 15 strains isolated from patients and on 15

TABLE 9.—*Comparison Between the Amount of Antibiotics Used on Two Wards Between 1954 and 1956*

Antibiotic	Amount Used, Gm.		Difference Between 3W & 3E, %
	2W (Medical)	3E (Surgical)	
Chlortetracycline	990	671	67
Oxytetracycline	680	832	122
Tetracycline	92	2,050	2,276
Chloramphenicol	2,445	2,248	93
Penicillin	6,937	3,667	75
Erythromycin	1,446	1,955	135
Streptomycin	165	794	401
Neomycin	1,386	758	83
Total	15,341	13,975	

strains isolated from staff members.* The commonest phage type was 44B in both groups, and no distinctions in phage types were apparent. Sensitivity studies of all of these cultures were performed and compared with cultures of staphylococci isolated from patients during the same weeks. Contrary to what other reports might have suggested, the strains from the house staff were more sensitive to penicillin and erythromycin than strains isolated from patients [19] (Table 8). The relatively small number of strains compared, however, should make one cautious in overinterpreting these results in spite of their apparent statistical significance.

In our opinion, the consideration of our data regarding staphylococci from carriers as compared to staphylococci isolated from patients suggests that, all in all, they are very similar. However, the fact that differences in sensitivities to antibiotics were found between groups of staphylococci isolated from infections and those isolated from carriers dictates caution in accepting fully conclusions regarding usage and resistance that were drawn mainly from studies on staphylococci isolated from carriers.[20,21] It may be that the relationship between the carrier state and infections seen on the wards is not as close as has been assumed.

(*e*) Strains of staphylococci isolated from another hospital in the community and studied by the tube dilution method were more sensitive to chloramphenicol, penicillin, and erythromycin than were the strains isolated at the Milwaukee County General Hospital during the same period (Table 8). Whether or not this was due to the fact that these antibiotics are the most popular at the latter hospital, we were not able to determine. The differences that were found were not of sufficient magnitude to change the relative order of activity of the antibiotics. Thus, the fact that these differences in staphylococcic sensitivities to antibiotics were found in hospitals within the same community might explain occasional discrepancies in reports from various parts of the country, but on the other hand, these differences, although significant, were not of great enough magnitude to negate the value that data from the one hospital might have in the determination of therapy for patients in another.

(*f*) Staphylococci isolated in January appeared to be more sensitive to chloramphenicol than were staphylococci isolated in August. There were no differences noted between these two groups in regard to their resistances to the other antibiotics. This could be due to a statistical quirk, or it might mean that things such as environmental temperatures do have importance in the evolutionary metabolic changes that take place in staphylococci. It should be remembered that many factors besides antibiotics are important in the environment of micro-organisms, and the finding that climate may have had an influence emphasizes the fact that we must take into consideration factors in evolution that may be far removed from the specific interests of any particular group of investigators when trying to evaluate effects of specific substances on population of micro-organisms.[22]

Comment

It is important to emphasize that the portions of this work concerned with influences of the usage of antibiotics on the resistance of staphylococci to them were done by statistical analysis of the differences between the means of the dilutions of the antibiotics necessary for inhibition. This was done in order to utilize as directly as possible the fact that the data were derived from approximately 75,000 separate cultures grown in the various antibiotics (each antibiotic was tested in 12 dilutions and each test included at least 10 antibiotics). The fact that the tube-dilution method of determining bacterial resistance to antibiotics lends itself particularly well to this sort of statistical analysis makes it particularly valuable for studies concerned with the

* The phage typing was done by Dr. Robert Wise, at his laboratory at Jefferson Medical College.

magnitude of changes in population that are by nature highly variable. The many different methods used to come to conclusions regarding changes in antibiotic sensitivities as related to antibiotic usage may explain different conclusions that have been drawn. No attempt will be made to review all the publications on this subject, but several generalizations seem fair to draw from the literature and from our studies. These are as follows.

(1) Our results are in essential agreement with studies that have been done in a similar manner.[23,24] Studies that have been reported to intimate a more profound influence of usage of antibiotics on the resistance of staphylococci to them have either been done mainly on staphylococci isolated from carriers within hospitals[20,21] or have used other methods of testing or of analyzing data.[25,26] Our data are also in agreement with our previous finding of relative stability of Gram-negative bacilli in regard to their sensitivity to antibiotics.[12]

(2) Penicillin sensitivity of organisms appears to be greater in staphylococci found outside the hospital environment.[16,17,26]

(3) Resistance of staphylococci to erythromycin appears to have increased rather markedly.[26-28]

(4) During the past few years, resistance to the tetracyclines, penicillin, and chloramphenicol has remained relatively stable.[24,26]

(5) It is our opinion that convincing definite proof that the use of antibiotics directly influences the sensitivity to antibiotics of staphylococci has not been offered. Our data show clearly that significant differences in antibiotic sensitivities can be demonstrated in groups of staphylococci exposed to identical antibiotic pressures but selected on unrelated bases, such as weather and geographic location. The rapid worldwide mutation of influenza virus provides an analogous example of a species change that occurred without a known influence by man, and it is not inconceivable that the same thing happened to the staphylococci in regard to their penicillin resistance.[29] Thus, our study, a review of the literature, and the fact that we are unaware of any such insignificant environmental pressure such as the relatively few tons of antibiotics that have been used in the world having ever before permanently changed the make-up of a species, lead us to believe that the burden of proof still rests with those who postulate that antibiotic usage has had a profound effect on the world of bacteria. The present importance of staphylococcic infections in general hospitals cannot be denied, however, and we are agreed that the magnitude of this problem may well be related in part to local effects which are due to antibiotic usage. The fact that hospital populations contain increasing numbers of patients who are particularly susceptible to infections due both to their ages and the illnesses which modern medicine is prolonging is in our opinion a more important cause of the staphylococcic problem. The variability of the staphylococci makes it mandatory for each hospital to determine any particular characteristics of staphylococci that it contains, but studies such as we have presented should be of aid in situations where facilities for precise large-scale studies are not available. It is apparent that any patient with a severe staphylococcic infection should have the benefit of a sensitivity test of his own organism, even though in severe cases it might be wise to begin therapy on the basis of statistical studies.

We feel that our study has certain implications regarding the problems of staphylococci in general hospitals, and as a result, we have adopted general policies which are based on our findings and those of others.[30] The policies and some of their rationale are as follows.

(1) "Wide-spectrum" antibiotics to which the majority of staphylococci in the hospital are resistant are not used unless absolutely necessary (Table 1).

(2) "Narrow-spectrum" antibiotics, such as penicillin or erythromycin, are used for pneumococcal and streptococcal infections and whenever else possible. This is done

because these agents do not disturb normal body flora, which in our opinion is the best protection against superinfection with the Staphylococcus.

(3) Treatment in severe staphylococcic infections is begun on the basis of our past experience, without delaying until the results of sensitivity tests are received. More than one agent is always used, and combinations are selected that will give a greater than 90% chance of being effective. Drugs are added every 18 hours until clinical response is obtained.

(4) Double masking, review of operating room technique and ventilation, isolation of severe staphylococcic infections, review of laundering technique and pick-up, tightening of wound-dressing techniques, and constant emphasis of the Staphylococcus as an important pathogen are other measures that have been adopted as a result of ours and other studies.[30] Staphylococcic carriers are not barred from the operating room or the wards because of the large number we have found. This facet of the problem is being met by emphasis on double masking. In the aggregate, it appears as if these measures are increasing the control of staphylococcic infections at the Milwaukee County General Hospital.

Summary and Conclusions

The antibiotic sensitivities of 500 strains of staphylococci isolated from patients at the Milwaukee County General Hospital were determined by the tube-dilution method of determining bacterial sensitivity to antibiotics. The amounts of antibiotics used on each ward of the hospital during a four-year period were determined. The results of the sensitivity tests were analyzed in regard to the relative activity of each antibiotic against staphylococci; the cross resistances of staphylococci to antibiotics; the optimal combinations of antibiotics; significant yearly changes in resistance to each antibiotic that occurred; differences in antibiotic sensitivity of groups of strains isolated from different sources, at different places in the hospital, and at different times of the year, and the relationships between antibiotic usage and staphylococcic resistance to antibiotics.

Our conclusions are that, as a species, staphylococci have been little influenced by antibiotics; that the vast majority of strains of staphylococci are sensitive to one or the other of several combinations of drugs that we have tabulated; that the problems of staphylococci in general hospitals may best be met by early diagnosis of infections, improvement of surgical and wound-dressing techniques, isolation of cases, and the usage of either narrow-spectrum antimicrobial agents, or in cases where they are necessary, wide-spectrum agents that are effective against staphylococci as well as the rest of the bacteria that make up normal body flora.

Dr. J. V. Talacko, Assistant Professor of Mathematics, Marquette University, gave advice regarding the statistical analysis.

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Erythropoietic Function in Uremic Rabbits

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Introduction

A moderate or often severe anemia of the normochromic normocytic type is usually present in patients with chronic renal disease. In 1950, Callen and Limarzi [1] made a careful hematologic study of 102 patients with renal disorders and reviewed the literature pertinent to this anemia. They concluded (1) that there was a functional suppression of the normoblastic elements of the bone marrow in renal disease with uremia and (2) that the degree of anemia appeared to be roughly proportional to the impairment in kidney function as measured by the level of nonprotein nitrogen in serum. Kidney pathology per se without retention of nitrogenous products in the blood was not associated with anemia. Recent studies of nephrectomized animals,[2,3] as well as studies of patients with uremia in whom the turnover of radioactive iron was measured,[4-6] have supported these conclusions.

Such studies have shown, in addition, that the anemia of uremia, as first pointed out by Emerson, in 1948,[7] must be hemolytic as well as aregenerative.[2,4-6,8-11] The life span of the red cells is shorter than normal, probably due to an extracorpuscular defect; there is a mild hyperbilirubinemia of the indirect type and an increased fecal excretion of urobilinogen, and following bilateral nephrectomy there is a rapid recession of the red cell mass.

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So far, it has been assumed that the aregenerative component of the anemia of chronic renal disease is caused solely by an inhibition of the bone marrow by toxic factors retained in the blood. However, other explanations are possible. Recently, it has been proposed that normal erythropoietic function is controlled, partly or wholly, by the level in the blood stream of an erythropoietic factor and that this level in turn is controlled by the tension of oxygen in a specific extramedullary site.[12] Any impairment of red cell production could, consequently, be the result of (1) a decrease in the level of erythropoietic factor in the blood stream or (2) a decrease in the responsiveness of the normoblastic elements of the bone marrow to erythropoietic factor.

In order to study the relationship between the aregenerative component of the anemia of uremia and this new concept of the regulation of red cell production the following experiments were carried out.

Materials and Methods

Adult white male New Zealand rabbits, weighing 2.2 to 3.2 kg. and fed rabbit pellets and water, were used throughout.

All operations were carried out with use of general anesthesia (1 to 1.5 ml. of 6% pentobarbital [Veterinary Nembutal] injected intravenously). The kidneys were approached either from incisions directly over the organs or from a single midline incision. The latter approach was used in the majority of the animals, since it was almost completely bloodless and since both kidneys could be approached through one incision. Postoperatively, each animal received 100 mg. of streptomycin and 50,000 units of crystalline penicillin subcutaneously. No instance of postoperative infection occurred despite only moderately sterile operative technique.

Anemia was induced by removing 17 to 20 ml. of blood per kilogram of body weight by heart puncture. In order to minimize hypovolemic shock,

the bleeding was promptly followed by an intravenous infusion of an equal volume of either 10% dextrose in saline with ephedrine sulfate added (15 mg. per kilogram) or 6% dextran in saline.

Peritoneal dialysis was performed in uremic animals once a day, beginning 48 hours after operation. Four hundred milliliters of Grollman's[28] dialyzing solution, to which dextrose had been added to a final concentration of 3%, was infused intraperitoneally and removed one to two hours later. Usually only one-half to three-quarters of the fluid could be removed.

The technique used in the bioassay of rabbit serum for erythropoietic factor has been described in detail in a previous publication.[14] In short, 50 ml. of serum to be examined was infused intravenously into normal recipient rabbits once a day for four days. After two preinfusion determinations, the reticulocyte counts were determined daily for six days and the reticulocyte response was used to evaluate the amount of erythropoietic factor in the serum.

Reticulocyte counts were made on dry coverglass smears, prestained with brilliant cresyl blue and counterstained with Wright's stain. One thousand red cells were counted. The average reticulocyte count in normal rabbits was 2.6% (ranging from 0.7% to 3.9%), with a standard deviation of 0.7%.

Hemoglobin concentrations were determined spectrophotometrically as oxyhemoglobin and averaged 12.9% in normal rabbits (ranging from 11.2 gm. to 14.5 gm. per 100 cc.).

In order to evaluate absolute changes in the number of reticulocytes per cubic millimeter, the reticulocyte count, expressed in per cent, was corrected whenever the hemoglobin concentration decreased significantly. Since the anemias encountered were always normochromic and since it was found that changes in hemoglobin corresponded almost exactly to changes in red cell counts, the corrections were calculated as follows:

$$\text{Corrected ret. } (\%) = \text{Observed ret. } (\%) \times \frac{\text{Observed hb.}}{\text{Base line hb.}}$$

Bone marrow aspirations were made from the tibia and cover-smear preparations prepared from bone marrow particles. One thousand nucleated cells were classified, and the number of normoblasts was expressed in per cent.

Nonprotein nitrogen determinations were made on serum, with use of Folin's method.[15] Values in normal rabbits ranged from 26 to 67 mg. per 100 cc. and averaged 38 mg. per 100 cc.

Results

A. Erythropoietic Response to Blood Loss

I. In Normal Rabbits.—Four rabbits were bled 18 ml. per kilogram. Their corrected reticulocyte counts remained unchanged for 24 hours and then rose and reached a peak, averaging 9.7% on the third day (Fig. 1). The normoblasts in the bone marrow increased from a mean of 30% to a mean of 50% on the fourth day.

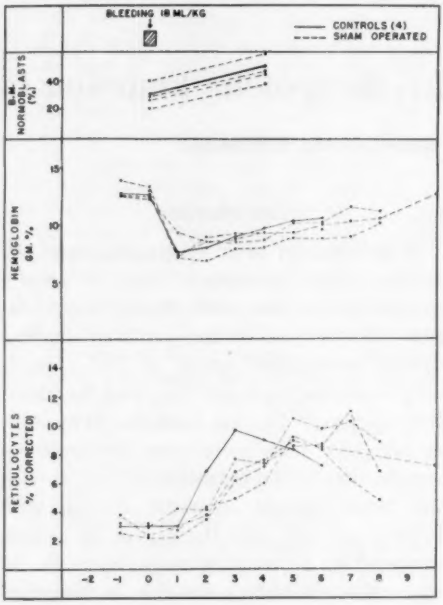

Fig. 1.—Hemoglobin concentration, corrected reticulocyte counts, and relative percentage of normoblasts in the bone marrow of rabbits bled 18 ml. per kilogram. Solid lines chart the mean response of four normal rabbits. Broken lines chart the response of rabbits, sham operated on just before the bleeding.

II. In Sham-Operated-On Rabbits.—In four rabbits a sham operation was performed. It consisted in unilateral nephrectomy and freeing of the contralateral kidney from its perirenal fatty envelope. Immediately after the operation the animals were bled 18 ml. per kilogram. The corrected reticulocyte counts remained unchanged for 48 hours and then rose and reached a peak on the fourth or fifth day (Fig. 1). On the fourth day, the normoblastic response of the bone marrow was identical to that observed in normal rabbits bled in the same manner.

III. In Bilaterally Nephrectomized Rabbits.—Bilateral nephrectomy followed by a

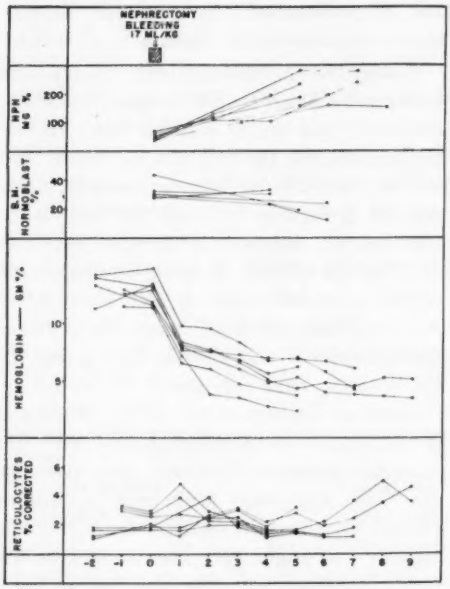

Fig. 2.—Hemoglobin concentration, corrected reticulocyte counts, relative percentage of normoblasts in the bone marrow, and nonprotein nitrogen in serum of rabbits nephrectomized just before a bleeding of 17 ml. per kilogram.

bleeding of 17 ml. per kilogram was performed in a number of rabbits. By means of daily peritoneal dialysis, it was possible to keep seven animals alive for a period sufficiently long (five days or more) to evaluate their erythropoietic response to the operation and blood loss.

From the day of operation the rabbits refused to eat or drink. Few had brief episodes of diarrhea but otherwise looked fairly well. The serum nonprotein nitrogen rose steeply despite peritoneal dialysis. However, dialysis once daily prolonged the life span by two to three days and induced a moderate reduction in nonprotein nitrogen.

After the initial drop in hemoglobin due to the hemorrhage, the hemoglobin concentration continued to fall until the animals died (Fig. 2). The reticulocyte counts, corrected for the drop in hemoglobin, remained low throughout except for a moderate agonal increase in two animals. The bone marrow revealed a complete lack of normoblastic response to the severe anemia. There was some fragmentation and irregularity of the nuclei of the orthochromatic normoblasts, but no major morphologic changes could be observed.

IV. In Uremic Rabbits with One Kidney.—Unilateral nephrectomy and contralateral ligation of the ureter followed by a bleeding of 17 ml. per kilogram was performed in a number of rabbits. By means of daily peritoneal dialysis two animals were kept alive for six and seven days, respectively. At autopsy the remaining kidney was found to be hydronephrotic but with normal-appearing parenchyma. The peritoneum appeared to be grossly normal.

Unilateral nephrectomy and contralateral division of the ureter with urine flowing freely into the peritoneal cavity was carried out in another group of animals. After a

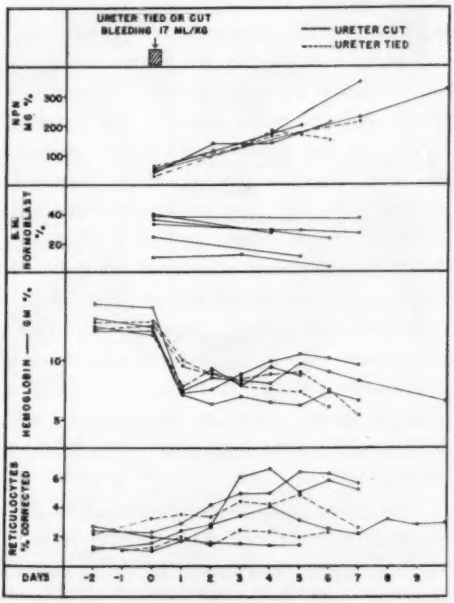

Fig. 3.—Hemoglobin concentration, corrected reticulocyte counts, relative percentage of normoblasts in the bone marrow, and nonprotein nitrogen in serum of rabbits operated on just before bleeding of 17 ml. per kilogram. Solid lines chart the responses of four rabbits on whom a unilateral nephrectomy and a contralateral division of the ureter were performed. Broken lines chart the responses of two rabbits on whom a unilateral nephrectomy and a contralateral ligation of the ureter were performed.

standardized bleeding of 17 ml. per kilogram and daily dialysis, four rabbits remained alive for five or more days. At autopsy the remaining kidney appeared normal. There was a slight fibrinous reaction of the peritoneum.

In a third group of rabbits, an attempt was made to connect the ureter with the lumbar vein by means of a polyethylene catheter. However, all animals died after a few days, and autopsy revealed clotted blood in the catheter, ureter, and renal pelvis.

The nonprotein nitrogen of all the animals operated on rose steeply as in the nephrectomized rabbits. After the initial posthemorrhagic drop in hemoglobin concentration, the hemoglobin value continued to fall in the two hydronephrotic animals (Fig. 3). In the animals in which the urine flowed into the peritoneal cavity, the hemoglobin concentration remained stationary or increased in the postoperative period. This difference between the two groups was probably caused by the severer dehydration which occurred in the animals with a divided ureter. In this group much larger amounts of fluid were recovered after peritoneal dialysis, often in volumes larger than those instilled. Blood-volume studies with use of Evans blue dye were attempted, but probably owing to the peritoneal dialysis were neither consistent nor strictly reproducible. However, there was a suggestively greater drop in plasma volume in the animals with a divided ureter than in the animals with unilateral hydronephrosis or with bilateral nephrectomy.

The reticulocyte counts increased moderately in two of the four animals with a divided ureter, increased slightly in one of the two with a ligated ureter, and were unchanged in the remaining three. Examination of the bone marrow revealed the same pattern in all six animals. Except for slight fragmentation and irregularity of the nuclei of the orthochromatic normoblasts, the marrows were essentially normal. As in the bilaterally nephrectomized animals, there was no evidence of a compensatory normoblastic response to the anemia.

Comment.—A standardized anemia was induced by blood loss in normal, sham-operated-on, and uremic rabbits. The effect of this anemia on the reticulocyte counts and on the normoblastic bone marrow tissue is charted in Figures 1, 2, and 3. Sham operation delayed the peak reticulocyte response by 24 hours without changing its magnitude. When the animals were rendered uremic by means of bilateral nephrectomy, the erythropoietic response was abolished altogether. In six animals rendered uremic by means of division or ligation of the ureter, three had no response to the hemorrhage and three had a slight response. However, this response was not associated with any significant change in the normoblastic bone marrow activity and might have been caused by the severe hemoconcentration observed in these three animals. Since the hemoglobin concentration was utilized in the calculation of the corrected reticulocyte percentages, hemoconcentration would result in an erroneously high reticulocyte level.

These findings suggest that both nephrectomized rabbits and rabbits with ligated or divided ureters fail to show a normal erythropoietic response to an acute hemorrhage and that this failure probably is related to the uremia rather than to the absence of kidney tissue.

B. Effect of Uremia on the Humoral Erythropoietic Factor

1. Reticulocyte Response of Normal Rabbits to Serum from Uremic Rabbits Subjected to Blood Loss.—Groups of normal rabbits had their renal function abolished by various operative procedures and were immediately bled 18 to 20 ml. per kilogram. An equal volume of 6% dextran in saline was infused in order to prevent hypovolemic shock. The animals were bled to death 12, 20, or 72 hours later and the serum bioassayed in normal recipient rabbits for erythropoietic factor. For each bioassay

serum was obtained from three to four animals and pooled.

The various operative procedures employed in rendering the rabbits uremic were as follows: (*a*) bilateral nephrectomy, (*b*) unilateral nephrectomy and contralateral ureter ligation, (*c*) bilateral ureter ligation, (*d*) interruption of the ureters or renal pedicles by means of "snares." In a preparatory operation one to two weeks before the actual experiment, polyethylene catheters (No. 50 or No. 90) were placed as loose snares around the ureters or renal pedicles. The end of the snares were brought out through the flanks and anchored to the skin. At the time of the experiment the snares were pulled tight and tied in a knot. In this way any nonspecific effect of anesthesia or surgical intervention was prevented.

(*a*) Donor Rabbits Maintained Anemic and Uremic for Twelve Hours: Mean hemoglobin concentration, 6.5 gm. per 100 cc.; mean nonprotein nitrogen, 72 mg. per 100 cc. Four normal rabbits from which one kidney had been removed previously and the other kidney was rendered nonfunctioning by means of a snare around the pedicle im-

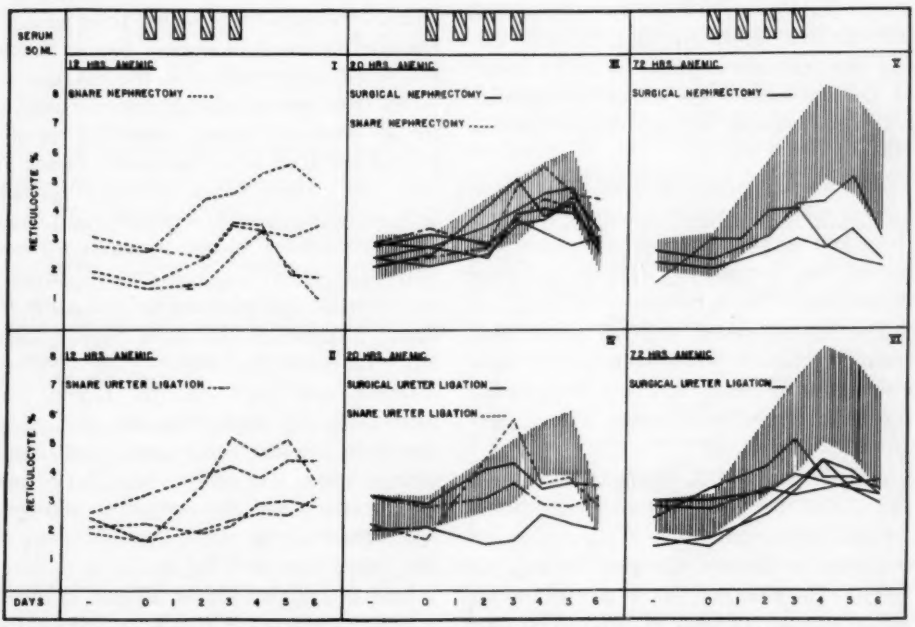

Fig. 4.—Reticulocyte counts of normal rabbits receiving 50 ml. of serum intravenously once a day for four days. I, broken lines chart the response of four normal rabbits to serum obtained 12 hours after snare nephrectomy and a bleeding of 20 ml. per kilogram. II, broken lines chart the response of four normal rabbits to serum obtained 12 hours after snare ureter ligation and a bleeding of 20 ml. per kilogram. III, solid lines chart the response of five normal rabbits to serum obtained 20 hours after bilateral nephrectomy and a bleeding of 20 ml. per kilogram. Broken lines chart the response of two normal rabbits to serum obtained 20 hours after snare nephrectomy and a bleeding of 20 ml. per kilogram. IV, solid lines chart the response of two normal rabbits to serum obtained 20 hours after ureter ligation and a bleeding of 20 ml. per kilogram. Broken lines chart the response of two normal rabbits to serum obtained 20 hours after snare ureter ligation and a bleeding of 20 ml. per kilogram. V, solid lines chart the response of three normal rabbits to serum obtained 72 hours after bilateral nephrectomy and a bleeding of 20 ml. per kilogram. VI, solid lines chart the response of five normal rabbits to serum obtained 72 hours after bilateral ureter ligation and a bleeding of 20 ml. per kilogram. The shaded areas in Sections III and IV represent the mean response ± one standard deviation of seven normal rabbits receiving serum from normal rabbits rendered anemic for 20 hours. The shaded areas in Sections V and VI represent the mean response ± one standard deviation of 25 normal rabbits receiving serum from normal rabbits rendered anemic for 48 hours or longer.

mediately before the acute hemorrhage (Fig. 4, Section I).

Four normal rabbits received serum from anemic rabbits in which both ureters were interrupted by means of snare (Fig. 4, Section II).

(*b*) Donor Rabbits Maintained Anemic and Uremic for Twenty Hours: Mean hemoglobin concentration, 6.2 gm. per 100 cc.; mean nonprotein nitrogen, 131 mg. per 100 cc. Five normal rabbits received serum from anemic rabbits in which bilateral nephrectomy was carried out immediately before the acute hemorrhage (Fig. 4, Section III).

Two normal rabbits received serum from anemic rabbits in which one kidney had been removed previously and the other kidney was rendered nonfunctioning by means of a snare around the pedicle immediately before the acute hemorrhage (Fig. 4, Section III).

Two normal rabbits received serum from anemic rabbits in which unilateral nephrectomy and contralateral ureter ligation was carried out immediately before the acute hemorrhage (Fig. 4, Section IV).

Two normal rabbits received serum from anemic rabbits in which both ureters were interrupted by means of snares immediately before the acute hemorrhage (Fig. 4, Section IV).

(*c*) Donor Rabbits Maintained Anemic and Uremic for Seventy-Two Hours: Mean hemoglobin concentration, 6.5 gm. per 100 cc.; mean nonprotein nitrogen, 200 mg. per 100 cc. Three normal rabbits received serum from anemic rabbits in which bilateral nephrectomy was carried out immediately before the acute hemorrhage (Fig. 4, Section V).

Five normal rabbits received serum from anemic rabbits in which bilateral ureter ligation was carried out immediately before the acute hemorrhage (Fig. 4, Section VI).

Figure 4, Sections I to VI, shows the individual daily reticulocyte percentages of the recipients of serum obtained from rabbits rendered both uremic and anemic. For comparison the shaded areas in Figure 4, Section III and IV, show the mean reticulocyte response ± 1 S. D. of 7 normal rabbits which had received similar amounts of serum from normal rabbits rendered anemic for 20 hours. The shaded areas in Figure 4, Sections V and VI, show the mean reticulocyte response ± 1 S. D. of 25 normal rabbits which had received similar amounts of serum from normal rabbits rendered anemic for 48 hours or longer.

The reticulocyte response to serum from animals rendered anemic and uremic for 20 hours was only questionably lower than the response to serum from normal animals rendered anemic for 20 hours. However, the reticulocyte response to serum from animals rendered anemic and uremic for 72 hours was significantly lower than the response to serum from normal animals rendered anemic for 48 hours or longer, suggesting an abnormal low level of erythropoietic factor in the serum. There was no definite difference between the response to serum obtained from animals rendered anemic by means of nephrectomy or by means of ureter ligation.

II. Reticulocyte Response of Normal Rabbits to Dialyzed Serum from Uremic Rabbits Subjected to Blood Loss.—Serum obtained three days after the rabbits had been bilaterally nephrectomized and made anemic by bleeding was pooled and dialyzed against saline. The dialysis was carried out for 48 hours, and the nonprotein nitrogen was reduced from more than 200 mg. to less than 15 mg. per 100 cc.

Bioassay of this serum in each of three normal rabbits gave the mean values for reticulocytes plotted in Figure 5. The absence of response indicates the same lack of erythropoietic activity in dialyzed as in undialyzed serum from uremic bled animals.

III. Reticulocyte Response of Normal Rabbits to Serum from Bled Normal Rabbits Dialyzed Against Uremic Serum.—Serum from normal rabbits rendered anemic by bleeding was dialyzed against serum obtained from nephrectomized and uremic rabbits. The dialysis was continued until the nonprotein nitrogen of the anemic serum

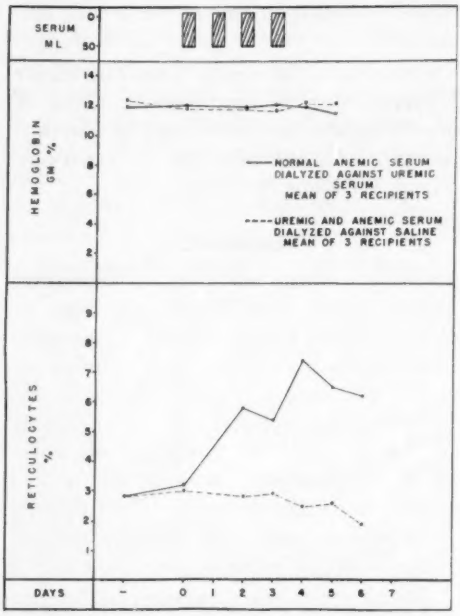
SERUM ML
HEMOGLOBIN GM %
NORMAL ANEMIC SERUM DIALYZED AGAINST UREMIC SERUM MEAN OF 3 RECIPIENTS
UREMIC AND ANEMIC SERUM DIALYZED AGAINST SALINE MEAN OF 3 RECIPIENTS
RETICULOCYTES %
DAYS

Fig. 5.—Hemoglobin concentrations and reticulocyte counts of normal rabbits receiving 50 ml. of serum intravenously once a day for four days. Solid lines chart the mean response of three normal rabbits to serum from normal bled rabbits after the serum was dialyzed against serum from uremic rabbits. Broken lines chart the mean response of three normal rabbits to serum from bilaterally nephrectomized bled rabbits after the serum was dialyzed against saline.

had increased from around 50 mg. to more than 150 mg. per 100 cc. The serum was then bioassayed in three normal rabbits. Figure 5 shows that the mean reticulocyte response resembles that which follows the infusion of serum from normal rabbits subjected to blood loss (shaded area in Fig. 4, Sections V and VI).

IV. Reticulocyte Response of Normal Rabbits to Bleeding and Infusion of Serum from Uremic Rabbits.—Three normal rabbits were bled 20 ml. per kilogram. Immediately after the hemorrhage they received the first of four daily infusions of 50 ml. of serum obtained from bilaterally nephrectomized and uremic rabbits. As controls, three other rabbits were bled in the same way but received daily infusions of serum obtained from normal rabbits. Figure 6 shows that the infusions of uremic serum did not significantly change the mean reticulocyte response induced by blood loss.

Comment.—Bioassay of serum from uremic rabbits subjected to blood loss suggested that anemic anoxia in animals rendered uremic for 72 hours by means of nephrectomy or ureter ligation fails to elicit a normal increase in erythropoietic activity of the serum. Serum from animals rendered uremic and anemic for 20 hours was only questionably less active than serum from normal animals rendered anemic for the same length of time.

Figure 5 shows that the lack of erythropoietic activity of the serum is not due to inactivation or destruction of erythropoietic factor by dialyzable elements in the uremic serum. Figure 6 shows that uremic serum in the amounts given did not interfere with the normal response to blood loss and, consequently, that nondialyzable elements in the uremic serum probably do not inactivate or

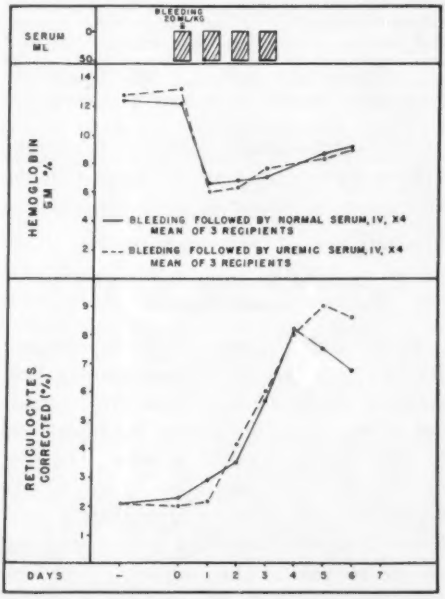
BLEEDING 20 ML/KG
SERUM ML
HEMOGLOBIN GM %
BLEEDING FOLLOWED BY NORMAL SERUM, IV, X4 MEAN OF 3 RECIPIENTS
BLEEDING FOLLOWED BY UREMIC SERUM, IV, X4 MEAN OF 3 RECIPIENTS
RETICULOCYTES CORRECTED (%)
DAYS

Fig. 6.—Hemoglobin concentrations and corrected reticulocyte counts of normal rabbits bled 20 ml. per kilogram infused with 50 ml. of serum once a day for four days. Solid lines chart the mean response of three rabbits receiving serum from normal rabbits. Broken lines chart the mean response of three rabbits receiving serum from uremic rabbits.

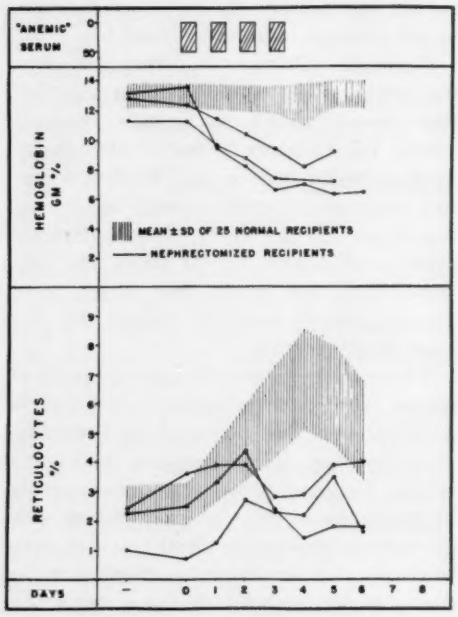

Fig. 7.—Hemoglobin concentrations and corrected reticulocyte counts of three nephrectomized rabbits receiving 50 ml. of serum from normal bled rabbits once a day for four days. Shaded area represents the mean response ± S. D. of 25 normal rabbits receiving serum from normal rabbits rendered anemic for 48 hours or longer.

destroy erythropoietic factor. The results suggest that there is an impairment in the production or release of erythropoietic factor in uremic rabbits.

C. Effect of Erythropoietic Factor on Uremic Rabbits

Reticulocyte Response of Nephrectomized Rabbits to Serum of Normal Rabbits Subjected to Blood Loss.—Serum from anemic but otherwise healthy rabbits was bioassayed in three nephrectomized rabbits. The first of four daily infusions of 50 ml. of the serum was administered immediately after bilateral nephrectomy had been performed on the recipient. The recipients were kept alive for five days or more by means of daily peritoneal dialysis. Figure 7 shows the corrected daily reticulocyte percentages in 3 recipients as well as the mean response of 25 normal recipients to similar serum. There was no significant increase in the reticulocyte counts in any of the uremic animals.

Comment.—The results shown in Figure 7 suggest that the erythropoietic tissue of uremic rabbits fails to respond in a normal manner to the administration of serum containing erythropoietic factor.

Comment

Under normal conditions a diminished oxygen supply to the tissues will cause an increase in red cell production. The mechanism responsible for this effect is unknown. It has been suggested either that the anoxia stimulates the erythropoietic tissue directly or that it causes the production elsewhere of an erythropoietic factor, which is then carried to the bone marrow. Until recently there was no convincing experimental evidence to support either of these theories.[12,16]

During recent years a number of different investigators have demonstrated that serum obtained from anemic animals is capable of inducing a reticulocyte response in normal recipients. It has been shown that this reticulocytosis reflects an increase in erythropoietic activity because, concomitantly, there is an increase in radioactive-iron incorporation into red cells.[17,18] Furthermore, prolonged administration of this serum to normal animals results in an appreciable increase in the number of circulating red cells in the hematocrit and in the relative number of normoblasts of the bone marrow.[19]

It has been proposed that the serum factor responsible for this stimulation of erythropoietic activity actually regulates red cell production. Recent studies have demonstrated the following facts to support such a proposal: (1) serum from animals exposed to air with a low oxygen concentration or to air under low barometric pressure has a similar erythropoietic activity[20,21]; (2) the activity of serum is inversely proportional to the oxygen tension of the air,[20] and (3) measurable erythropoietic activity appears in the serum 4 to 6 hours after exposure to anemic or hypoxic anoxia,

reaches a maximum 16 hours later, and persists as long as the anoxia is maintained. After termination of the anoxia the activity of serum returns to normal within three hours.[22] These findings indicate that the erythropoietic factor is of physiologic significance and support the hypothesis that tissue anoxia controls red cell production by means of a humoral erythropoietic factor.

With this hypothesis in mind it was decided to study the pathogenesis of the anemia of uremia. It has been shown that several factors are involved in the production of this anemia. First, red cells have a shortened life span in uremia. Second, gastrointestinal bleeding is a frequent complication. Third, there is a functional suppression of red cell production, with a failure to compensate for the accelerated blood destruction and increased blood loss. It was hypothesized that the bone marrow failure, probably the most important of the pathogenic factors, could be caused by an impairment in the production or action of the erythropoietic factor.

Recently Jacobson and co-workers [23] have reported that rats rendered uremic by means of nephrectomy failed to produce erythropoietic factor in response to an acute hemorrhage, while similarly challenged rats with ligated ureters produced significant amounts of this factor. Their conclusions were that the erythropoietic factor was manufactured or activated in the kidneys. The anemia of uremia could, consequently, be caused by impaired production or activation of erythropoietic factor in the damaged kidney. Several reports of polycythemia associated with tumors of the kidneys [24-26] appear to support this hypothesis, since the polycythemia could have been caused by pathologic overproduction of erythropoietic factor in the renal tumors.

However, certain observations are not in accord with this hypothesis. Callen and Limarzi [1] found that even severe renal disease with uremia is rarely associated with normoblastic hypoplasia of the bone marrow, and in their series of 102 uremic patients not a single case had erythroid bone marrow aplasia. Furthermore, a reduction in the oxygen supply to the kidneys, as obtained by placing a Goldblatt clamp around the renal artery, has never been reported to result in polycythemia. Only two reports actually have data on the hemoglobin concentrations and hematocrits, but in both reports a reduction in blood supply to the kidneys resulted in anemia rather than polycythemia.[27,28]

In the present study it was first shown that seven rabbits made uremic by means of bilateral nephrectomy failed to respond to a standardized hemorrhage with an increase in erythropoietic activity, as measured by reticulocyte counts and relative number of normoblasts in the bone marrow. However, neither did the erythropoietic activity decrease, as would have been expected if the kidneys controlled red cell production through production of erythropoietic factor.

Six rabbits made equally uremic by means of unilateral nephrectomy and contralateral ligation or division of the ureters failed similarly to respond in a normal way to a standardized hemorrhage. The relative number of bone marrow normoblasts remained unchanged in all rabbits, and in three animals the reticulocyte counts did not change significantly. A slight increase in the corrected number of reticulocytes was found in the other three animals. However, severe dehydration and hemoconcentration was present in these three animals, resulting in a low correction factor and, consequently, in erroneously high corrected reticulocyte counts.

The absence of a significant erythropoietic response to an acute hemorrhage both in rabbits without kidneys and in rabbits with divided or ligated ureters suggests that the uremic state common to both inhibits red cell production.

Second, it was demonstrated that serum from uremic animals challenged with a standardized hemorrhage failed to show a normal increase in erythropoietic activity after 72 hours of anemic anoxia. However,

serum obtained after 20 hours of uremia and anemia was only slightly less active than serum from normal animals rendered anemic for 20 hours.

These findings indicate that severe uremia is associated with a significant suppression of erythropoietic activity of serum, while early mild uremia only is associated with a slight and questionable suppression. There was no significant difference in erythropoietic activity between serum from rabbits rendered uremic by means of nephrectomy and serum from rabbits rendered uremic by means of ureter ligation.

Third, it was demonstrated that the low erythropoietic activity of serum from rabbits rendered uremic and anemic was due to a decreased production or release of the erythropoietic factor rather than to inactivation of the factor in the blood stream. Finally, it was shown that the erythropoietic tissue in uremic animals did not respond to the stimulating effect of serum with known erythropoietic activity.

Severe uremia undoubtedly influences the metabolism of every living cell. Consequently, a derangement in the production and in the effect of erythropoietic factor could be anticipated. It is of interest that the aregenerative anemia which accompanies a localized sterile abscess is associated, although to less degree, with the same dual deficiency, a decreased production or release of erythropoietic factor and a decreased erythropoietic response to this factor.[29]

As long as the site of origin of the erythropoietic factor is unknown, these findings cannot be explained. It is, of course, possible that the erythropoietic factor is produced as a metabolic waste product by the dividing and maturing erythropoietic stem cells and that the low serum level of this factor in uremia and in inflammation only reflects the decreased activity of these cells. However, erythropoietic factor has been demonstrated in serum from anemic rabbits with bone marrow activity completely suppressed by mechlorethamine hydrochloride (nitrogen mustard).[27] Consequently, it seems more likely that the erythropoietic factor is produced in an extramedullary locus and that it is of physiologic significance.

This would mean that uremia, in addition to inducing hemolysis, blood loss, and depression of the metabolism of erythropoietic cells interferes with the process by which the tissue tension of oxygen normally controls the production of erythropoietic factor.

Summary

The erythropoietic response to a standardized hemorrhage was evaluated in normal and uremic rabbits by means of reticulocyte counts, bone marrow examinations, and bioassay for erythropoietic serum factor.

Rabbits made uremic by means of bilateral nephrectomy, or by unilateral nephrectomy with contralateral ligation or division of the ureter, failed to respond to a standardized bleeding of 17 or 18 ml. per kilogram with a normal increase in reticulocytes and bone marrow normoblasts.

Serum from rabbits rendered uremic for 72 hours by nephrectomy or ureter ligation failed to show a normal increase in erythropoietic activity following a standardized hemorrhage. This deficiency was found to be due to a lack of production or release of erythropoietic factor rather than to its inactivation in the blood stream.

Uremic rabbits failed to respond with a reticulocytosis to the infusion of serum with known erythropoietic activity.

In conclusion, these findings indicate that the anemia of uremic rabbits is related to the metabolic changes associated with uremia rather than to the presence or absence of kidney tissue and that it is associated both with a decreased production or release of erythropoietic factor and a decreased erythropoietic response to this factor.

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Bone Marrow Hemosiderin and Ferrokinetics Patterns in Anemia

I. Pernicious Anemia

RALPH O. WALLERSTEIN, M.D., and MYRON POLLYCOVE, M.D., San Francisco

Increased tissue iron in patients with pernicious anemia was first described in 1910, by Ryffel,[1] who used autopsy material. Increased hemosiderin in bone marrow aspirates was demonstrated in 1948, by Rath and Finch,[2] who showed that such a rise in bone marrow hemosiderin is common to all anemias except that of iron deficiency and that it is due largely to a shift of iron from the red blood cells to storage.[3] Beutler recently reviewed quantitative aspects of iron stores.[4] The present study of marrow hemosiderin deals with its pattern of distribution rather than its total amount. Whenever possible observations on its microscopic appearance were correlated with data obtained from ferrokinetic measurements.

Methods and Material

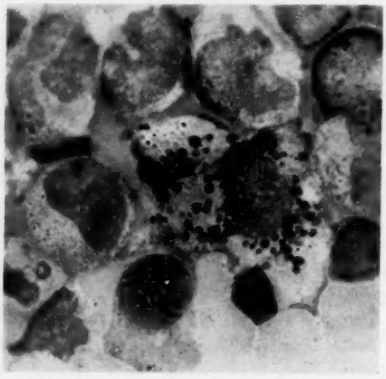
Bone marrow aspirations were performed using a 16 gauge needle * and a 10 ml. syringe previously rinsed with a 10% solution of edathamil disodium (Disodium Versenate). The aspirate was ejected on an ordinary microscope slide or cover slip and the liquid part blotted off. After drying, the slide was dyed with Prussian blue stain prepared as follows: 4 gm. of potassium ferrocyanide were diluted to 20 ml. with distilled water; concentrated hydrochloric acid was added until a permanent precipitate formed. The dried smears were then covered with filtrate for 30 minutes. Only glassware made iron-free by washing in potassium dichromate and sulfuric acid solution was used. When combined Wright's and Prussian blue stains were employed to localize hemosiderin granules more precisely, the slides were first dyed with Wright's stain in the usual manner and after drying were stained for iron as described above. Best results were obtained with minimum amounts of concentrated hydrochloric acid in the preparation of the Prussian blue stain.

Total amounts of hemosiderin granules and amorphous material were graded separately from 0 to 4+, according to their appearance in tissue particles. Normal values range from 1 to 2+. Differential counts of granular size were made with the aid of a micron measuring disk † which had a 5 mm. scale. Subdivisions of the scale, calibrated with a counting chamber, measured 0.6μ at magnification $\times$ 1000. The field of vision was reduced by a mask with a central hole of 5 mm., and at least 200 granules were counted on each slide. Granules of less than 0.6μ in diameter were not included in the counts (see below). Ferrokinetics measurements were made as previously described.[5,10]

The diagnosis of pernicious anemia was established by the characteristic blood and bone marrow morphology and by the presence of

† B&L 31-16-01.

Fig. 1 (Patient 7).—Pernicious anemia. Note granules of similar size in histiocyte cytoplasm. Wright and Prussian blue stains; $\times$ 900.



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From the Children's Hospital Research Laboratory, San Francisco, and the Donner Laboratory, University of California, Berkeley, Calif.

* Bechton, Dickinson & Co., 485 LNR.

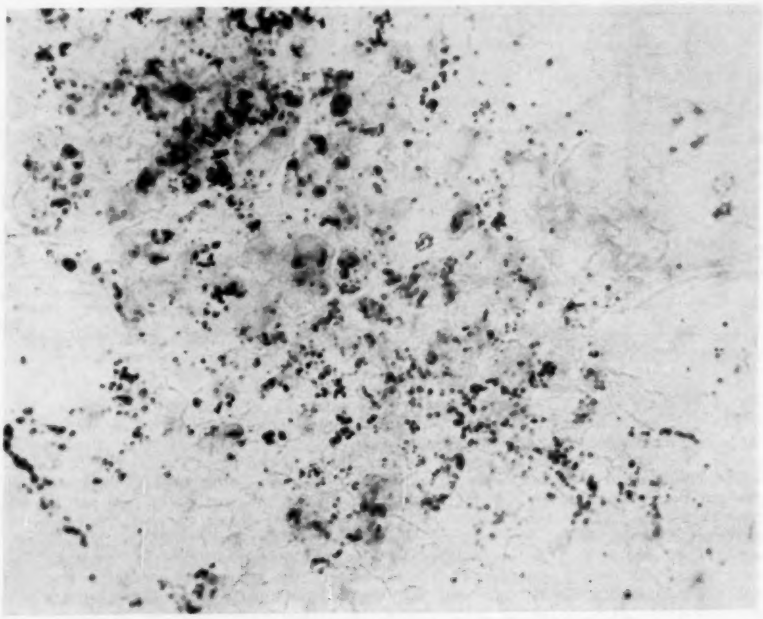


Fig. 2 (Patient 2).—Pernicious anemia. Note homogeneity of granules. Prussian blue; × 420.

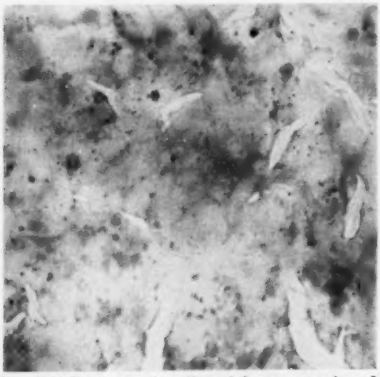


Fig. 3 (Patient 6).—Pernicious anemia. Note homogeneity of granules. Prussian blue; × 420.

histamine-fast achlorhydria. The diagnosis was confirmed by a reticulocyte response to cyanocobalamin (vitamin B_{12}) and eventually by return to normal blood values as a result of further therapy.

Hemolytic anemia was diagnosed either by the usual laboratory findings of accelerated blood destruction—reticulocytosis, bone marrow erythroid hyperplasia, increased amounts of bilirubin and stool urobilinogen, or radioiron studies demonstrating increased hemoglobin synthesis and decreased erythrocyte survival. Sickle-cell anemia was diagnosed by the sodium metabisulfite method and paper electrophoresis.

The diagnosis of aplastic anemia was based on diminished number of bone marrow erythrons and diminished hemoglobin synthesis, with reduced Fe^{59}

TABLE 1.—*Data on Patients with Pernicious Anemia*

Patient	Bone Marrow Hemosiderin							Clinical Data ‡			
	Granular						Amorphous				
	Diameter * Distribution, %					Total Amount	Total Amount †	Diagnosis	Age Yrs	Hb gm/100 ml	TF
	1-2	2-3	3-4	4-5	5						
1	81	16	2.5	0.5	--	4+	O-T	P. A.	60	2.7	0
2	99	1	--	--	--	4+	O-T	P. A.	70	5.0	0
3	98.5	1	0.5	--	--	4+	O-T	P. A.	67	4.0	0
4	99	1	--	--	--	3+	O-T	P. A.	64	9.7	0
5	86	13	1	0.5	0.5	3+	O-T	P. A.	66	7.0	0
6	81	16.5	2.5	--	--	3+	1+	P. A.	56	5.8	0
7	99	1	--	--	--	2-3+	O-T	P. A.	70	7.5	0
8	93.5	5.5	1	--	--	2+	O-T	P. A.	72	7.5	0

* Diameter in micrometer subdivisions; each equal to 0.6μ at magnification × 1000.
† T indicates trace.
‡ TF indicates previous transfusions; P. A., pernicious anemia.

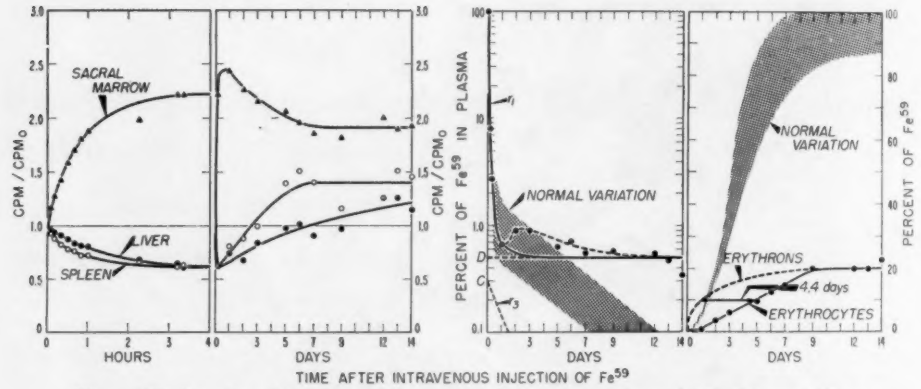


Fig. 4 (Patient 6).—A 56-year-old woman; weight, 71 kg.; hemoglobin, 5.8 gm/100 ml.; pernicious anemia. On the right are shown in vitro measurements of plasma and erythrocyte radioiron content obtained after intravenous injection of Fe^{59}-transferrin (incubated fresh plasma.[10]) The lack of continued decrease of radioiron in the plasma after the first day indicates abnormal reentry of radioiron. From Slopes r_1 and r_3, Intercept C, and total plasma iron it can be shown that of 144 mg. of iron leaving the plasma daily, 130 mg. is fixed in marrow erythrons.[11] The rate of fixation is shown by the dotted curve labeled "erythrons." This corresponds to daily synthesis of 38.3 gm. of hemoglobin. Relating this to total hemoglobin of 262 gm., a mean erythrocyte life span of seven days is obtained. In vivo measurements over sacral marrow, liver, and spleen are shown on the left. Radioactivity at each site is related to the extrapolated counting rate obtained at zero time when all radioiron is in circulating plasma. Initial accumulation of radioiron occurs only in marrow and is abnormally rapid. Marrow release of radioiron, however, is delayed and incomplete. This suggests a functional erythropoietic block with erythron destruction in the marrow and relatively little effective production of circulating reticulocytes and erythrocytes. This is confirmed by the markedly subnormal net incorporation of radioiron in circulating erythrocytes and the prolonged mean erythron hemoglobinization time of 4.4 days (normal, 1.4±0.2 days). In addition to marked marrow destruction of erythrons, there is also moderate splenic sequestration and destruction of erythrocytes demonstrated by the secondary accumulation of radioiron in the spleen. This occurs as radioiron is released from marrow and appears in circulating erythrocytes. The slow progressive increase of hepatic radioactivity is characteristic or iron exchange between plasma and storage, not erythrocyte sequestration.

uptake by bone marrow and circulating erythrocytes.

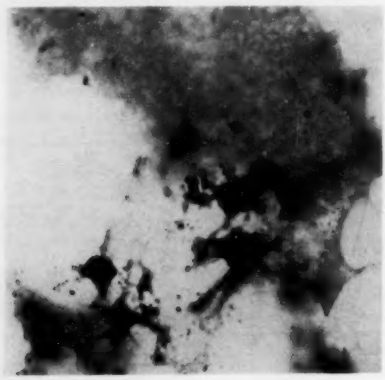
Results

Increased amounts of bone marrow hemosiderin were found in most of the patients, regardless of diagnosis. It occurred in two distinct morphologic forms, granular and amorphous. Granules varied in size from 0.6μ to more than 3μ in diameter. They usually stained intensely blue with the Prussian blue reaction; occurred singly, in aggregates of more than 20, or surrounded by by amorphous material, and seemed to be contained in histiocytes (Fig. 1).

Amorphous hemosiderin formed strands, streaks, irregularly shaped patches, and meshworks measuring from 3μ to more than 50μ in length, apparently intracellular and staining paler than the granular hemosiderin with the Prussian blue reaction; but it had the same spectroscopic pattern.‡

In addition to these two forms of hemosiderin storage, tiny blue dots of less than 0.6μ in diameter were seen in the

Fig. 5 (Patient 16).—Aplastic anemia. Note amorphous masses surrounded by a few granules. Prussian blue; × 420.



‡ Performed by Dr. Nicholas Petrakis.

TABLE 2.—*Data on Patients with Other Anemias*

Patient	Bone Marrow Hemosiderin: Granular Diameter * Distribution, % 1-2	2-3	3-4	4-5	5+	Granular Total Amount	Amorphous Total Amount	Clinical Data † Diagnosis	Age, Yr.	Hb, Gm./100 Ml.	TF
9	56.5	25.5	10.5	2	5.5	4+	1+	R. A.	79	10.6	28
10	40	31.5	15	2.5	6	3+	1+	SCA	4	6.6	0
11	37.5	17.5	15.5	15.5	14	3+	3+	Acute H. A.	8	7.5	0
12	81.5	11	4	2	1.5	2+	O-trace	CNSHA	21	12.3	0
13	77	10.5	7	4.5	1	2+	1+	Leukemia	59	6.5	0
14	52	21.5	9	2	10.5	2+	2+	SCA	1½	7.2	0
15	50.5	19.5	18.5	1	11.5	2+	4+	RMF	68	7.7	0
16	Too few to count					O-trace	4+	Aplastic A	31	6.0	30

* Diameter in micrometer subdivisions; equal to 0.6μ at magnification of × 1000.
† TF indicates previous transfusions; R. A., refractory anemia with erythroid hyperplasia; SCA, sickle-cell anemia; CNSHA, congenital nonspherocytic hemolytic anemia; Leukemia, lymphatic leukemia, chronic; RMF, anemia with relative bone marrow failure.

cytoplasm of late erythroblasts, normoblasts, reticulocytes, and some adult cells; these correspond to the sideroblasts of several authors.[6-9] They occurred in all forms of anemia except iron deficiency anemia and were not included in the differential counts.

Bone marrow hemosiderin in patients with pernicious anemia was predominantly granular (Figs. 2 and 3). The granules were of similar size and frequently were packed tightly together in grape-like clusters, which superficially looked like single large clumps; but individual granules could be discerned on careful focusing. Most of the granules were less than 2μ in diameter; granules larger than 3μ were rare, even in severe anemia. Amorphous material was scanty (Table 1).

The ferrokinetics pattern of Patient 6, with pernicious anemia, is shown in Figure 4. Similar patterns have been found in other patients with pernicious anemia.[5]

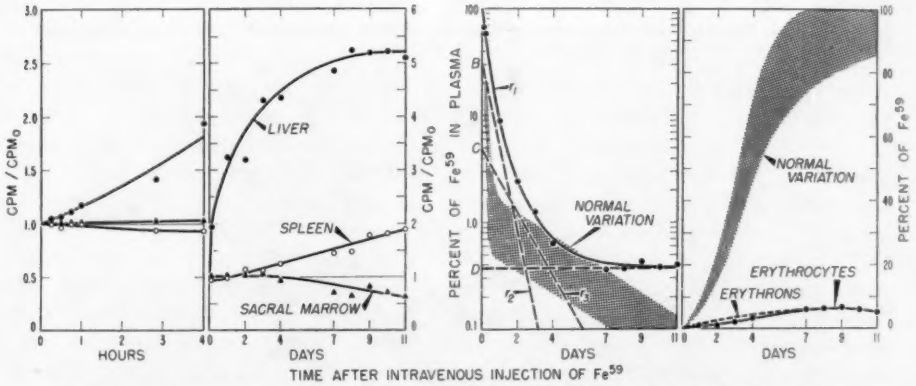


Fig. 6 (Patient 16).—A 31-year-old man; weight, 81 kg.; hemoglobin, 10.0 gm/100 ml.; erythroid hypoplasia. In vivo measurements over sacral marrow, liver, and spleen show that as radioiron leaves plasma, significant accumulation occurs only in liver and, to less degree, spleen. The absence of hepatic and splenic release of radioiron suggests that no extramedullary erythropoiesis is present in these organs. The slow progressive increase of hepatic and splenic radioactivity is the result of iron exchange between plasma and storage. The lack of significant marrow accumulation and release of radioiron indicates that marrow erythropoiesis is negligible. This is confirmed by net incorporation in circulating erythrocytes of only 6% of plasma radioiron. From Slopes r_1, r_2, r_3, Intercepts B and C, incorporation of radioiron into erythrons, and total plasma iron, it can be shown that of 25 mg. of iron leaving plasma daily, 1.3 mg. is fixed in marrow erythrons. This corresponds to daily synthesis of 0.4 gm. of hemoglobin, 0.08 gm. per liter of blood (normal, 1.3±0.2 gm.). When this is related to the daily decrease in total hemoglobin, 8.0 gm., and total initial hemoglobin, 519 gm., a mean erythrocyte life span of 62 days is obtained, approximately that of fresh compatible transfused erythrocytes.

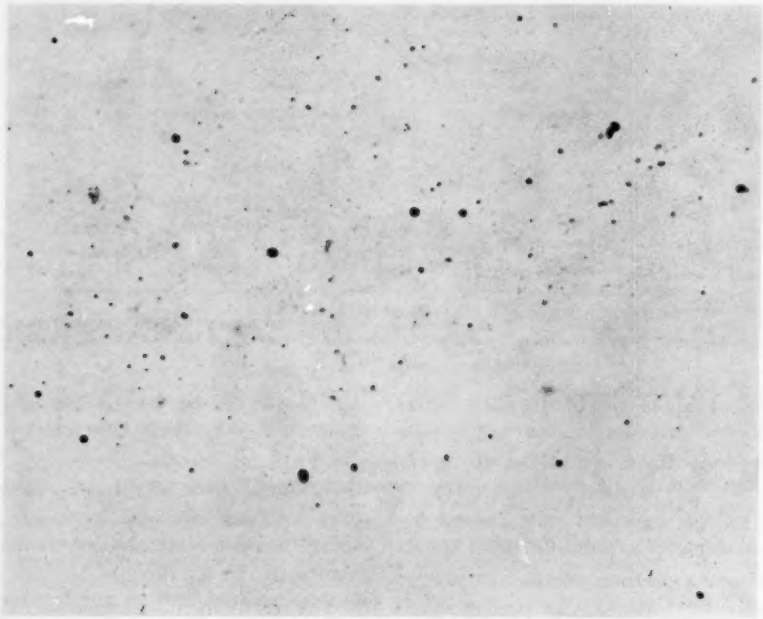
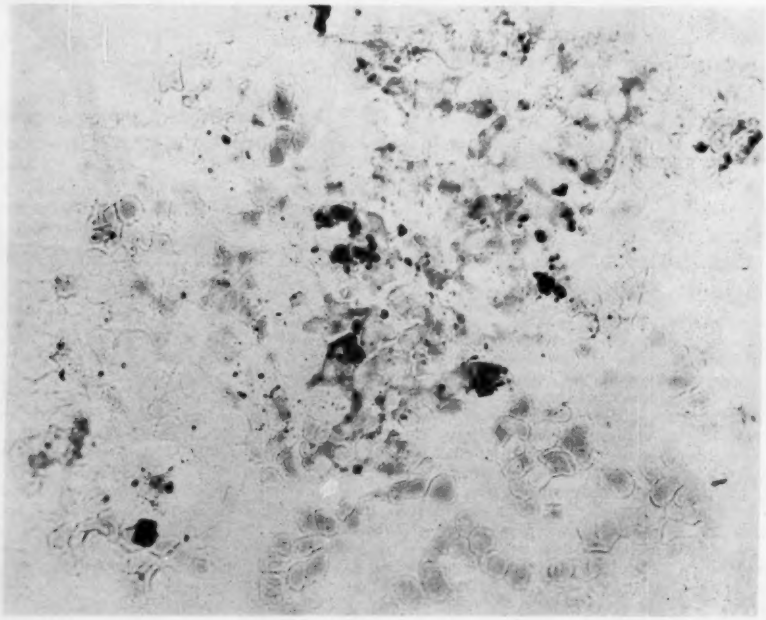


Fig. 7 (Patient 12).—Congenital nonspherocytic anemia. Note variation in size of granules, lack of amorphous hemosiderin. Prussian blue; × 420.

Fig. 8 (Patient 11).—Acute hemolytic anemia. Note variation in size of granules, lack of amorphous hemosiderin. Prussian blue; × 420.



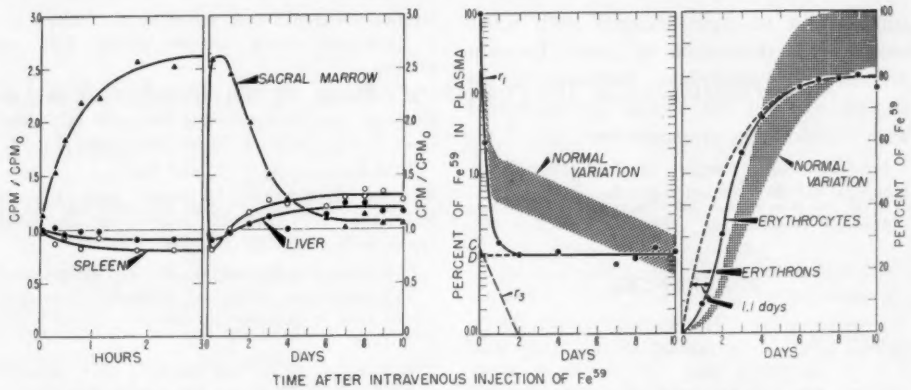


Fig. 9 (Patient 12).—A 31-year-old man; weight, 62.7 kg.; hemoglobin, 12.3 gm/100 ml.; hereditary nonspherocytic hemolytic anemia. The lack of continued decrease of radioiron in the plasma after the second day indicates abnormal reentry of radioiron. In vivo measurements show secondary splenic and hepatic accumulation of radioiron with rapid and virtually complete marrow release of radioiron. Correspondingly rapid and somewhat decreased net incorporation of radioiron in circulating erythrocytes occurs simultaneously. This suggests that reentry of radioiron into plasma proceeds from early destruction of newly formed circulating erythrocytes. From Slopes r_1 and r_3, Intercept C, and total plasma iron, it can be shown that of 94 mg. of iron leaving the plasma daily, 91.6 mg. is fixed in marrow erythrons. The rate of fixation is shown by the dotted curve labeled "erythrons." This corresponds to daily synthesis of 26.9 gm. of hemoglobin. Relating this to total hemoglobin of 428 gm., a mean erythrocyte life span of 16 days is obtained. Mean erythron hemoglobinization time of 1.1 days is normal (normal range, 1.4±0.2 days).

In patients with aplastic anemia, most of the hemosiderin was in the amorphous form; there were relatively few granules (Fig. 5 and Table 2). The ferrokinetics pattern of Patient 16, with aplastic anemia, is given in Figure 6.

In patients with hemolytic anemia several hemosiderin patterns were observed. Granules predominated but varied much in size; many were large, measuring more than 3μ in diameter (Figs. 7 and 8). Amounts of amorphous material varied but were never greater than amounts of granular hemosiderin. The ferrokinetic pattern of Patient 12, with congenital nonspherocytic anemia, is shown in Figure 9.

Comment

This study describes two morphologically different forms of hemosiderin storage. A granular pattern was usually associated with conditions of bone marrow hyperplasia and rapid iron turnover. In addition to the cases described here we have observed granular patterns in thalassemia major, even after many transfusions, in erythroblastosis, and in leukemia. An amorphous pattern was observed in aplastic anemia and decreased iron turnover. Similar patterns were encountered in patients with anemia associated with azotemia, metastatic carcinoma, and infections. A subsequent report will correlate hemosiderin and ferrokinetics patterns in these groups.

Ferrokinetics measurements in patients with pernicious anemia give evidence that though effective erythropoiesis is markedly decreased, iron turnover within the marrow is greatly increased. Data obtained from hemosiderin determinations are consistent with such a concept. The homogeneity of the granular size is not readily explained. It may mean that the nucleated red cells of the bone marrow are hemolyzed at similar stages of their development, a maturation arrest in the sense that their metabolic defect does not permit them to survive beyond a certain point in their development.

Summary

Bone marrow hemosiderin patterns are analyzed in eight patients with pernicious

anemia and in eight patients with other anemias. A distinction is drawn between granular and amorphous hemosiderin. In several patients the results are compared with ferrokinetics measurements.

Mr. Marshall Douglas, of Letterman Army Hospital, did the photomicrography.

Medical Arts Building, 2000 Van Ness Ave. (9).

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The Epidemiology and Serology of Rheumatoid Arthritis

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Striking progress has been made in the definition of the problem of rheumatoid arthritis by the recent application of epidemiological [1-3] and serological [4] techniques which, not very long ago, would have been considered inappropriate for this field. This report will present control and case data which were obtained by the application of two of the newer serological methods,[5,6] as well as preliminary analysis of a survey of the prevalence of certain signs and symptoms of arthritis in Syracuse, N. Y. The relation of some of the prevalence to serological data also will be discussed.

Materials and Methods

A. Latex Fixation Test.—The test described by Singer and Plotz[6] as modified by them to use glycine-buffered saline at pH 8.2 as the diluent and with the exception noted below was employed throughout.

1. Latex: Polystyrene-latex particles * of 0.81μ diameter were used as the antigen vehicle. According to the authors' instructions, 2 ml. of the stock polystyrene-latex suspension containing 11% solids should be added to 20 ml. of water and this suspension then filtered through Whatman No. 40 paper. When 0.1 ml. of this suspension is added to 10 ml. of the glycine buffer, the light transmission at 650 mμ should measure 5%. In our experience, this almost always came out as 32% on the Colemar Junior Model A machine which we have available. Four similar spectrophotometers yielded equivalent results. By means of a series of trials, it was determined that 15% transmission yielded a satisfactorily dense suspension for performing the test. This concentration was regularly achieved by diluting 0.5 ml. of the stock solution with 15 ml. of glycine-buffered saline.

2. Buffer: Glycine-buffered saline was made according to the instructions of Singer[6] by adding 2.5 ml. of 1 N NaOH to 975 ml. of distilled water in which 7.5 gm. of glycine had been dissolved. After adjusting the pH to 8.2, NaCl, 10 gm., was added and the volume brought to 1000 ml. The pH was checked and the buffered saline transferred to the refrigerator for storage until used.

3. γ-Globulin: Stock γ-globulin solution was made by dissolving 0.5 gm. of lyophilized human Fraction II † in 100 ml. of glycine-buffered saline. This was stored in the refrigerator until needed.

4. Procedure: All sera were stored in the frozen state until tested by being screened at dilutions of 1:10, 1:20, and 1:40. In accordance with the authors' directions, agglutination in a serum dilution of 1:20 or greater was considered to be a positive test. In no instance was a serum positive at 1:10 and negative at 1:20. Sera from patients with rheumatoid arthritis which failed to yield positive reactions in the three screening tubes were retested in twofold dilutions to 1:5120. None was found to have a prozone. In other respects, the procedure was as recommended.[6]

B. Bentonite Test.—A copy of the recommended procedure for performing this test was supplied by the authors.[7] Their recommendations were observed, except that the same screening serum dilutions that had been adopted for the latex fixation test were employed. Although the authors state that the test should be considered positive if a 2+ or greater reaction occurs in a serum dilution of 1:32, for comparative purposes we selected a 2+ or greater reaction in the 1:20 dilution as being positive. A second change was that the test was conducted in Linbro clear plastic Disposo-Trays, in place of rings on a slide. These are convenient to handle and can be used over again,

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* Dr. John Vanderhoff, Dow Chemical Co., Midland, Mich., supplied the latex particles.

† Supplied by E. R. Squibb & Sons, at the request of the American Red Cross Blood Program.

but the tray has somewhat deep wells. It would be preferable to have wells of about half their capacity.

We were able to confirm the authors' experience[7] that when negative sera are diluted beyond 1:1000 one may note 1 and 2+ reactions. These do not alter the conclusions that these sera are negative. Such reactions could be interpreted as indicating that the difference between a positive and a negative serum is that the positive serum reacts at lower dilutions, as though some inhibitory substance which is present in "normal" serum is diminished in the sera of patients with rheumatoid arthritis.

C. Serum Samples.—1. "Normal" Sera: These were collected during an extensive study of the "normal" Syracuse, N. Y., population. Although its basic purpose was to obtain control data to be used in the evaluation of toxoplasma serological results in patients with uveitis, other tests have been applied to the specimens to acquire additional information and to evaluate the groups further. This population will be defined more completely elsewhere, but it may be stated at this time that they represent "normal" upper and lower income ambulant populations. Their selection was determined by their living in a randomly selected home, on a randomly selected street. The groups of streets were chosen from those of the city's census tracts which would provide persons of contrasting income levels.

Although a questionnaire was completed on the individual members of each family, no more than two (one parent and one child) were asked to submit to bleeding and skin testing with toxoplasma antigen. A total of 2857 questionnaires were completed, and 803 of these persons were bled. An additional 108 questionnaires have been completed from the families of patients with uveitis. In the present report, the analyses are limited to those persons whose blood was tested. The more detailed analysis will deal with the entire sample and consider such factors as age, sex, economic level, and previous illnesses.

2. Other Sera: (*a*) Rheumatoid arthritis. These sera were obtained from patients seen by us in the Medical Center (many specimens were obtained from patients at the affiliated Veterans' Administration Hospital,‡ so there is a preponderance of male subjects). All were considered to have active rheumatoid arthritis.

(*b*) Nonrheumatoid arthritics. These sera were from patients who had been seen by us and whose clinical diagnoses were other than rheumatoid arthritis.

(*c*) Rheumatic sera. These specimens were routine bleedings from 114 patients who attend our Rheumatic Fever Prophylaxis Clinic. Although they are a reasonably well population (except for the presence of rheumatic heart disease or a history of rheumatic fever), they are not as "normal" a population as that which was bled during the city-wide survey.

‡ The staff of this hospital provided the serum samples.

TABLE 1.—*Infrequency of Positive Latex-Fixation Tests in 801 "Normal" Persons*

Age Group Yr.	Sample	Male		Female		Positive Latex Fix.	
		No.	%	No.	%	No.	%
0–4	46	22	48	24	52	0	0
5–9	77	42	55	35	46	0	0
10–19	103	49	48	54	52	0	0
20–29	107	48	45	58	54	0	0
30–39	157	75	48	82	52	0	0
40–49	118	43	36	75	64	0	0
50–59	83	40	48	43	52	0	0
60–69	68	35	51	33	49	1 *	2
70–79	38	20	53	18	48	1 †	3
80–89	4	2	50	2	50	0	0
Totals	801	376	47	425	53	2	0.25

* Represents 3% of males.
† Represents 5% of males.

Results

In Table 1 are summarized the results obtained with the latex-fixation test when the sera of 801 "normal" persons were examined. Only two positive tests were encountered, and both of these were in men who were 60 or more years of age. Thus, in this "normal" population, 0.25% of persons yielded a positive latex-fixation test. This is remarkably low and leads to the conclusion that in Syracuse, N. Y., at least, positive reactions are unusual in the population at large.

When the latex-fixation test was applied to 114 members of the Rheumatic Fever Prophylaxis Clinic, six, or 5.3%, yielded positive tests (Table 2). All of these were males, despite the fact that the sample contained almost twice as many females as males. One of the six men has definite rheumatoid arthritis and another is a strong possibility. If these two subjects are removed from the totals, then the incidence of positive reactors becomes 3.6%, or 12 times that which was observed in the "normal" ambulant population.

Sera, also, were tested from 225 persons with a variety of diseases not considered to be rheumatoid arthritis. Patients with an-

TABLE 2.—*Occurrence of Positive Latex-Fixation Tests Among 114 Members of a Rheumatic-Fever Prophylaxis Clinic*

Age Group, Yr.	Total No.	Male		Female		Remarks (Positive Reactors Only)
		Pos.	Neg.	Pos.	Neg.	
5–9	2	0	0	0	2	
10–19	24	1	12	0	11	Has had acute RF and glomerulonephritis; no evidence of arthritis; test minimal positive
20–29	19	0	4	0	15	
30–39	30	1	7	0	22	Male has RHD only; reaction strongly positive
40–49	25	2	7	0	16	1 man considered to be a "possible" rheumatoid arthritic; 2d male has RHD and no signs or symptoms suggestive of RA; his test is the more positive of the 2
50–59	9	2	2	0	5	1 man has RHD and RA, while the other has RHD only
60+	5	0	1	0	4	
Totals	114 *	6	33	0	75	

* Males indicate 34.2% of subjects, but 18% of them were positive; females, 65.8% of subjects, but none were positive. Among all subjects, 5.3% were positive.

kylosing spondylitis are included in this group because we have considered this to be a different disease from rheumatoid arthritis. Among the 225 persons, 13 (5.8%) gave positive reactions (Tables 3 and 4). This is almost identical with the uncorrected results observed with the sera from the Rheumatic Fever Prophylaxis Clinic patients and emphasizes the differences in "normal" rates which may be arrived at when different kinds of control populations are selected for this purpose.

TABLE 3.—*Distribution of Nonrheumatoid Arthritic-Positive Latex Reactors by Age and Disease*

Category	Age Group					Totals
	20–29	30–39	40–49	50–59	60+	
Rheumatic fever						
Acute		2				2
Rheum. ht. dis.		2	1	1	1	5
Gout			2			2
Lupus ery. dis.	1 *					1
Epider. carcinoma			1			1
Prim. atyp. pneum.		1 †				1
Tuberculosis		1 †				1
Totals	1	6	4	1	1	13

* This patient is a woman; all others are men.
† Same person as *.

Fifty-eight per cent of the patients with rheumatoid arthritis (Table 5) were found to have positive tests. When reconsidered on the basis of sex, 63% of the males and 51% of the females were positive. This difference may be more apparent than real because 15 of the 37 females were less than 20 years of age. Only three of the 43 males were under 20 years. If one reexamines the figures in this light, it will be seen (footnote, Table 5) that in the over-20-year age groups, 68% and 73% of males and females,

TABLE 4.—*Distribution of Nonrheumatoid Arthritic-Negative Latex Reactors by Age, Sex, and Disease*

Category	Age Group														Totals		Grand Totals
	5–9		10–19		20–29		30–39		40–49		50–59		60+				
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
Rheumatic fever																	
Acute	2	0	2	3	7	1	8	2	3	2	0	0	0	0	22	8	30
Rheum. ht. dis.	0	0	0	1	8	0	16	1	5	1	1	1	3	0	33	4	37
Gout	0	0	0	0	1	0	8	0	9	0	2	1	3	0	23	1	24
Osteoarthritis	0	0	0	0	0	0	0	0	2	2	2	0	6	2	10	4	14
Reiter's	0	0	0	0	2	0	0	0	0	2	0	0	0	0	2	2	4
Ankyl. spondylitis	0	0	0	0	3	0	3	0	1	0	0	0	1	0	8	0	8
Lupus ery. dis.	0	0	0	1	0	1	0	4	0	0	0	0	0	0	0	6	6
Scleroderma	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1
Arthritis																	
Allergic	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1
Traumatic	0	0	0	1	0	0	1	2	0	0	1	0	0	0	2	3	5
Infectious	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	2	3
Tuberculous	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1
Unknown	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1
Chorea	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	2
Card.-vasc.	0	0	0	0	0	0	7	0	2	0	4	0	3	0	16	0	16
Nonsp. pericarditis	0	0	0	0	0	0	5	0	0	0	0	0	0	0	5	0	5
Genitourinary	0	0	0	0	2	0	2	0	4	0	0	0	0	0	8	0	8
Infections, misc.	0	1	1	0	9	0	4	2	3	0	1	0	1	0	19	3	22
Allergic	0	0	0	0	3	0	1	0	0	0	0	0	0	0	4	0	4
Misc. dis.	0	0	0	0	5	1	6	1	6	0	0	0	0	1	17	3	20
Totals	3	1	3	7	42	3	62	14	36	7	11	2	17	4	174	38	212

TABLE 5.—*Positive Latex-Fixation Tests in Patients with Rheumatoid Arthritis*

Age Group, Yr.	Male			Female			Totals		
		Positive			Positive			Positive *	
	No.	No.	%	No.	No.	%	No.	No.	%
0–9	1	0	0	4	0	0	5	0	0
10–19	2 (1) †	0	0	11	3	27	13	3	23
20–29	1 (1)	0	0	3 (1)	2	67	4	2	50
30–39	11	6	55	5 (1)	4	80	16	10	63
40–49	7	6	86	9 (1)	7	78	16	13	81
50–59	14	10	71	3 (1)	2	67	17	12	71
60+	7	5	71	2 (1)	1	50	9	6	67
Totals	43 (2)	27	63	37 (5)	19	51	80	46	58

* If patients under and over 20 years of age are considered separately, then the following figures are obtained:

Positive	>20 yr.	<20 yr.
Male	27/40=68%	0/3=0%
Female	16/22=73%	3/15=20%
Totals	43/62=69%	3/18=17%

† Numbers in parentheses indicate patients whose clinical diagnosis was considered to be "questionable rheumatoid arthritis." They are not included in the calculations, and none had a positive test. Had their tests been positive, they still would have been considered separately.

respectively, were positive. The combined total then becomes 69%, while only 17% of the group under 20 years of age were positive.

There were seven persons who were parenthetically included in Table 5 because their diagnosis of rheumatoid arthritis was highly questionable on the basis of clinical data. None of these had a positive latex test, and none was included in the final tallies. It is of interest, though, that they were all negative.

The sera of 28 patients with uveitis were available, and when these were examined (Table 6) it was found that one female yielded a positive test. Since she was in the latter portion of the second trimester of a pregnancy (acute flare-ups of her disease usually come during pregnancy), it was thought that pregnancy might have played a role in her response to the latex test.

TABLE 6.—*Latex-Fixation Test Results Obtained Among Twenty-Eight Patients with Uveitis*

Age	Male		Female	
	No.	Pos.	No.	Pos.
17–29	3	0	4	1 *
30–39	5	0	5	0
40–49	3	0	3	0
50+	3	0	2	0
Totals	14	0	14	1

* This 27-year-old patient had no history or findings suggestive of arthritis. Her uveitis flares with each pregnancy. Since she was in the late second trimester at the time her serum was obtained, the sera of 13 pregnant females (late second or third trimester) were examined, but none yielded a positive latex-fixation test.

Accordingly, sera were obtained from 13 pregnant women, each of whom was in the latter part of her pregnancy, but none of these was found to have a positive test. The patient with uveitis has no signs or symptoms suggestive of rheumatoid arthritis, so the explanation for her positive latex fixation test remains obscure. It does appear, however, that a positive latex-fixation test is uncommon in granulomatous uveitis.

The serum of each of the rheumatoid arthritis patients was examined with C-reactive protein (CRP) antiserum § at the time when the latex fixation test was performed. In addition, each patient had an erythrocyte sedimentation rate, measured by the Wintrobe method, on the same day. It has been reported that patients with rheumatoid arthritis may have positive CRP reactions, and it is common experience that they frequently have elevated sedimentation rates. Tables 7 and 8 summarize these experiences, with the former being concerned with those patients who had a positive latex-fixation test and the latter, with those whose tests

§ Schieffelin & Company, New York.

TABLE 7.—*CRP and ESR Reactions of Rheumatoid Arthritis Patients with Positive Latex-Fixation Tests*

Age Group, Yr.	CRP		ESR		CRP Pos. & ESR Pos.	CRP Pos. & ESR Neg.	CRP Neg. & ESR Pos.	CRP Neg. & ESR Neg.
	Pos.	Neg.	Pos. *	Neg.				
10–19	3	1	4	0	3	0	1	0
20–29	1	0	1	0	1	0	0	0
30–39	3	7	7	3	2	1	5	2
40–49	8	2	9	1	7	1	2	0
50–59	9	2	11	0	9	0	2	0
60+	6	0	6	0	6	0	0	0
Totals	30	12	38	4	28	2	10	2

* Corrected Wintrobe greater than 10 mm. per hour.

TABLE 8.—*CRP and ESR Reactions of Rheumatoid Arthritis Patients with Negative Latex-Fixation Tests*

Age Group, Yr.	CPR		ESR		CRP Pos. & ESR Pos.	CRP Pos. & ESR Neg.	CRP Neg. & ESR Pos.	CRP Neg. & ESR Neg.
	Pos.	Neg.	Pos. *	Neg.				
0–9	3	2	5	0	3	0	2	0
10–19	3	4	6	1	3	0	3	1
20–29	1	2	2	1	1	0	1	1
30–39	5	2	7	0	5	0	2	0
40–49	0	3	2	1	0	0	2	1
50–59	5	0	5	0	5	0	0	0
60+	2	0	2	0	2	0	0	0
Totals	19	13	29	3	19	0	10	3

* Corrected Wintrobe greater than 10 mm. per hour.

were negative. The results are tabulated according to the ages of the test subjects. Since the numbers are relatively small, there appears to be no value in analyzing them by sexes. It will be noted that among the 42 patients whose latex-fixation reaction was positive, the sedimentation rate was more often elevated than was the CRP reaction positive. Although there was agreement in 28 of the patients, 10 persons had abnormal sedimentation rates and negative CRP reactions. There were two instances in which both tests were negative and two others where the CRP was positive and the sedimentation rate, normal.

In Table 8 are summarized the CRP reactions and sedimentation rates observed in 32 patients whose latex-fixation tests were negative. Both tests were positive in 19 persons, but in 10 others the sedimentation rate was abnormal and the CRP reaction, negative. Neither test was positive in three instances.

If one summarizes these two Tables, it will be noted that among 74 patients with active rheumatoid arthritis the sedimentation rate was abnormal 67 times and the CRP test was positive 49 times. Both tests were positive in 47 persons, but there were 20 instances in which the CRP reaction was negative and the sedimentation rate, elevated. This experience suggests that the CRP reaction may be positive in about two-thirds of persons with active rheumatoid arthritis while the sedimentation rate, as employed in this study, was abnormal approximately 90% of the time. Rapid sedimentation rates were about evenly distributed between patients with positive and negative latex-fixation reactions, but the CRP reaction was more often positive when the latex-fixation test was positive.

Bozicevich and co-workers [7] have recently described a bentonite-flocculation test for detecting the presence of rheumatoid arthritis. The authors made the procedure available to us, and we have examined the sera of 57 persons by both the bentonite and the latex-fixation methods. There were 26 instances in which both tests were positive, 20 patients in whom both tests were negative, 9 patients who had a positive latex-fixation test and a negative bentonite test, and 2 persons who had negative latex-fixation and positive bentonite tests (Table 9). Among 34 patients with rheumatoid arthritis, the tests agreed 28 times, but there were 5 instances in which the latex fixation was positive and the bentonite, negative, and 1

TABLE 9.—*Comparative Results Obtained with the Latex and Bentonite Tests Among Fifty-Seven Persons*

Diagnostic Group	Both Tests		Latex Pos. & Bentonite Neg.	Latex Neg. & Bentonite Pos.
	Neg.	Pos.		
Rheumatoid arthritis				
Definite	10 *	18 †	5 ‡	1
Doubtful	1	0	0	0
Rheumatic fever				
Acute	1	1	0	0
Rheum. ht. dis.	0	3	2	0
Lupus ery. dis.	2	1	0	1
Reiter's	2	0	0	0
Gout	0	1	1	0
Ankyl. spondylitis	1	0	0	0
Osteoarthritis	1	0	0	0
Uveitis	0	1	0	0
Normal	2	1	1	0
Totals	20	26	9	2

* Three are children.
† One is a child.
‡ One is a child.

person in whom the reverse was true. The patient with disseminated lupus erythematosus was positive by both tests. She is a young female who had been diagnosed as having rheumatoid arthritis four years before the onset of LED. A sample of her serum stored since 1953 gave the same reactions as that obtained in 1957. Arthritis continues to be an important factor in her disability.

Of the two tests, we found the latex-fixation simpler and quicker to perform. These points of superiority are substantial and, when added to the apparent greater sensitivity of the latex method, combine to make this the preferable method at this time.

Epidemiology

Cobb and his colleagues [8] have recommended three questions ("Index of Rheumatoid Arthritis") as being of value for arriving at an indication of the prevalence of arthritis through mass interviews: (*a*) Do you wake up with stiffness or aching in your joints or muscles? (*b*) Have you ever had arthritis or rheumatism? (*c*) Have you ever had swelling in any joints? These questions were asked of the approximately 3000 persons who were contacted in our community survey, and while they will be analyzed by age, sex, economic status, and other aspects in complete detail elsewhere, a preliminary analysis is tabulated in Table 10. The information is limited here to that from 678 persons, all of whom were 10 or more years of age and whose serum sample was incorporated into the "normal" control population which was studied by the latex-fixation reaction. The data are broken down by sex, but there is no breakdown by age or economic status. Both of the latter factors appear to be quite important, for the frequency of positive replies is substantially greater among women than among men, increases with age, and is commoner in the lower than in the upper income level. For our immediate purposes, it will be noted that more women than men replied in the affirmative to the three questions and, further, that about 5% of the females and 2% of the males answered "yes" to all three questions. Conversely, 66% of the women deny ever having had any of these symptoms or signs, in contrast with such replies from 83% of the men. That the answers to these questions do not necessarily signify rheumatoid arthritis confirmable by a positive latex-fixation reaction is apparent when one compares Tables 1 with 10.

TABLE 10.—*Replies of 678 "Normal" Persons Subsequently Examined by the Latex-Fixation Test to Questions Concerned with Arthritic Complaints*

Question *	Male		Female		Totals	
	No.	%	No.	%	No.	%
A	18	6	35	10	53	8
B	12	4	21	6	33	5
C	1	0.3	9	3	10	2
D	9	3	26	7	35	5
E	2	0.6	5	1	7	1
F	4	1	9	3	13	2
G	7	2	20	5	27	4
H	258	83	242	66	500	74
No. questioned	311	46	367	54	678	100

* A: Do you wake up with stiffness or aching in your joints or muscles? (Yes).
B: Have you ever had arthritis or rheumatism? (Yes).
C: Have you ever had swelling in any joints? (Yes).
D: Yes to both A and B.
E: Yes to both A and C.
F: Yes to both B and C.
G: Yes to A, B, and C.
H: No to A, B, and C.

The problem of bursitis is an interesting one, but there has been little information concerning its prevalence. As a bit of pilot information, histories of previous bursitis were sought from 818 persons who were

TABLE 11.—*Incidence of History of Previous Bursitis Elicited from 818 "Normal" Persons*

Sex	Age Group, Yr.														Totals		Total No.	Pos., %
	0–9		10–19		20–29		30–39		40–49		50–59		60+					
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.		
Male	0	151	0	57	1	46	1	64	3	42	9	23	0	19	14	402	416	3.4
Female	0	147	0	73	0	30	6	45	5	42	6	28	0	20	17	385	402	4.2
Totals	0	298	0	130	1	76	7	109	8	84	15	51	0	39	31	787	818	3.8

contacted during the latter part of the community survey (Table 11). It will be noted that no one under 20 years of age stated that he had ever had an episode of bursitis. The number with a positive history increases steadily by decades, except that none of the 60-year-old group could recall ever having had such an attack. This sample is somewhat small, and the result may be misleading, for the question was so worded that it should have produced an accumulation of positive answers in the older age groups. Only 3.8% of these persons said that they had had an attack of bursitis at sometime or another, but positive histories were more frequent in women than in men.

Fifty-three persons with a history of bursitis have been questioned more intensively, and it was learned that fourteen had noted the onset of an attack during the fall; eighteen, during the winter; seventeen, during the summer, and only four, during the spring. Thirty-one of fifty-two persons stated that one shoulder had been involved, while twelve gave a history of both shoulders having been affected; thus, forty-three of fifty-two attacks occurred in the shoulder joints. These should be considered as only preliminary pilot data, and much more information is required before any definite conclusions can be reached.

Comment

Although the various agglutination reactions which have been recommended for use as diagnostic aids in rheumatoid arthritis recently have been reviewed by Ziff [4] and need not be considered in detail at this time, several aspects appear worthy of emphasis. In Ziff's [4] summaries of the results obtained by different investigators with the different serological procedures which have been applied to patients with rheumatoid arthritis, positive results in controls have been reported to vary from 1% to 30% in different samples. The larger series seem to indicate that about 5% to 7% of their controls have been positive by the sheep-cell agglutination test. Plotz and Singer [9] found that 3% of their controls yielded positive latex-fixation tests, in comparison with 71% of patients with rheumatoid arthritis.

It will be noted from the data which we have presented that different rates of positive reactions may be observed in control groups which have been selected by different means. Only 0.25% of the ambulant Syracuse population (nonhospitalized and not selected because they were ill with some disease other than rheumatoid arthritis) was found to be positive, but in the sample composed of those suffering from some illness other than rheumatoid arthritis the rate increased to 5.8%. This is comparable with the results obtained from the examination of persons attending our Rheumatic Fever Prophylaxis Clinic, where 5.3% yielded positive tests. In other words, the ideal control population for the evaluation of serological procedures in rheumatoid arthritis does not appear to be persons who are selected, basically, because they are sick with some illness other than clinical rheumatoid arthritis. An interesting confirmation of this experience appears in a report by Cobb et al.,[1] in which it seems that when the sera of 223 persons who had been selected on the basis of a positive response to their "Index of Rheumatoid Arthritis" during a community survey were examined with the sensitized sheep-cell agglutination test,[10] only one positive result was obtained. This yields a rate of 0.45%, in comparison with the 0.25% observed among our ambulant controls.

Reports of previous experiences with the latex-fixation test are limited and, unfortunately, not entirely appropriate to the present discussion. The most comparable study is that of Plotz and Singer,[9] who found that 71% of patients with rheumatoid arthritis had positive reactions. If children are omitted from our data then our results would be similar, namely, 69%. The incidence of positive reactions varies from 1% to 5% among their various control groups.

More recently, Rothermich and Philips [11] have described their experiences with a modification [12] of the latex-fixation test which differs importantly from that devised by Singer and Plotz and used by us. In this modification, the γ-globulin which is used for the sensitization of the latex particles is liquid γ-globulin, which was reported by Singer and Plotz [5] to be less desirable than dried Fraction II. The liquid material has been unsatisfactory in our hands, too. The second important difference is that Rheins and Rothermich used latex particles with a diameter of 1.17μ in place of the recommended [5] 0.81μ. Third, they use borate-buffered saline, which Singer and Plotz abandoned for the glycine buffer employed in the present study. Last, the modified latex-fixation reaction is performed by a drop method, and the period of incubation is shortened considerably. This makes a direct comparison of our results with theirs almost impossible. These authors report [11] that 84% of their rheumatoid arthritis patients have positive tests, whereas patients with other kinds of rheumatic diseases have a rate of 3.6% and those with nonrheumatic diseases, a rate of 5.4%. The results in the last two control groups are almost identical with those observed by us. The greatest discrepancy appears to be among patients with rheumatoid arthritis, which may reflect the differences in technique.

We have no information on which to base any comparison between the results obtained by us and others with the bentonite test. On the basis of the data which were presented in this report, we would have to conclude that the latex-fixation test is superior. The results obtained with one pair of sera are of special interest. Two specimens obtained at an interval of five weeks from the same person were examined by both the latex and the bentonite methods. Both specimens were positive by the former method, but the first serum was negative by the bentonite procedure and the second was definitely positive. Although the sample which was negative by the bentonite procedure was positive by the latex test, it yielded a lower-titered reaction than did the second specimen. We have no explanation for this discrepancy, but it would appear to be useful to examine a second specimen from a patient who yields a negative test when the clinical data suggest that the reaction will be positive. We also found the latex test to be technically simpler to perform and thus superior at the practical diagnostic level.

The prevalence data which are reported herein are of considerable interest. If one assumes that a "yes" answer to all three questions is strongly suggestive of a history of rheumatoid arthritis, it would seem that 4% of the "normal" persons who had serological examinations had had an episode of rheumatoid arthritis. About two and one-half times as many women gave this history as did men. Cobb concluded [1] that about 2.7% of his Pittsburgh test population over the age of 14 years had strong evidence of definite rheumatoid arthritis. He, too, found a higher rate in women than in men. Our data compare favorably with his, although the numbers are quite small.

Because of the nature of the questions, one would expect the rates to increase with age, since the questions are concerned with past history and positive histories should accumulate with age. This is true of both Cobb's and our data. The most disturbing feature is that the historical information is not supported by serological experience. This was not only true in our study but also appears to be present in Cobb's experience. It would seem proper to conclude that one needs more alteration than is indicated by the replies to the "Index" questions before a serological reaction, like the latex-fixation test, will become positive.

Bursitis is an intriguing problem for which there is very little real information except clinical impression. The data contained in the present report are certainly of a preliminary nature, but they do suggest behavior which is not entirely consistent

with the commonly expressed notion that attacks of this kind are precipitated by trauma. This is only one of many questions in the rheumatic disease field which need formally collected information to permit the beginnings, at least, of reasonable definitions of the problems which remain to be investigated.

Summary

Sera from four different kinds of populations were examined with the latex-fixation test: (*a*) Only 0.25% of a "normal" ambulant population had positive tests; (*b*) 5.3% of persons with a history of rheumatic fever had positive tests; (*c*) 5.8% of persons suffering from a variety of diseases other than rheumatoid arthritis were positive; (*d*) 58% of rheumatoid arthritics (69% of adults) had positive latex-fixation tests.

The latex-fixation and bentonite tests were applied to the sera of 57 persons, and the former was found to be more sensitive and easier to perform.

Among 678 "normal" persons, 74% answered "no" to three questions concerned with previous rheumatic disease. Four per cent answered "yes" to all three, and among these the rate in women was more than twice that in men. There was no positive correlation between affirmative replies to the questions and the presence of a positive latex-fixation test.

Mrs. Colleen Trody, Mrs. Sondra Kluger, and Kenzo Kuroda aided in various aspects of this study.

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Nitrogen Metabolism After Portacaval Shunts in Patients with Cirrhosis

I. Effects of the Operation upon the Blood "Ammonia" Concentration

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With the Technical Assistance of Marjorie Knowlton, Barbara Stowers, and Anna Marie Link

From the standpoint of eradication of esophageal varices an end-to-side portacaval shunt is the most efficient method of treating portal hypertension.[1,2] On the other hand, complete diversion of portal blood from the liver has been demonstrated to result occasionally in neurological abnormalities similar to those occurring spontaneously in patients with severe chronic liver disease, and these changes have been related to the nitrogen content of the diet and to the concentration of "ammonia" in the blood.[3-6] The relative importance of portacaval shunts on the one hand and hepatocellular failure on the other in producing the neurological syndrome associated with severe liver disease has been discussed extensively.[6,7]

Measurements of the changes in blood "ammonia" concentration immediately after portacaval shunts were undertaken to assess the contribution of the shunting of blood to the syndrome of liver failure. It was found that a rise in blood "ammonia" concentration occurred consistently during the few days following operation, but the transient nature of the rise suggested that postoperative liver-cell failure played a major part in its causation.

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Patient Material

Clinical data on the 13 patients studied are summarized in Table 1. Their ages ranged from 23 to 62. Twelve had cirrhosis, and Patient 9 had a normal liver and a thrombosed portal vein. Nine of the patients with cirrhosis had histories of excessive alcohol intake. Seven had had one or

TABLE 1.—*Clinical Data on Thirteen Patients*

Patient	Preoperative Data						Duration of Operation, Hr.	Portal Vein Pressure	
	Age, Yr.	History of Hemorrhages	Ascites	BSP Retention *	Serum Albumin †	Globulin †		Before Shunt	After Shunt
1 ‡	23	5 mo. before	No	8	3.8	3.3	?		
2	42	5 mo. before	No	28	3.3	4.3	4	430	260
3	39	None	Mild	31	4.0	3.7	3 ½	420	205
4 §	50	Emergency operation	No		2.4	2.6	3	Not measured	
5	45	None	No	25	3.1	4.8	4	420	240
6	62	3 wk. before	No	29	2.2	5.4	3 ¾	Not measured	
7	38	None	Mild	14	4.4	3.0	3 ¾	300	150
8	24	3 mo. before	Moderate	7	4.4	4.4	2 ¾	348	150
9 §	25	None	No	9	4.2	3.7	3 ½	310	245
10	41	2 mo. before	No	11	3.7	4.4	5	495	205
11	35	None	Slight	44	2.6	4.4	2	480	280
12	47	6 mo. before 1 mo. before	No	15	4.1	3.9	5 ½	440	110
13	45	None	Moderate	10	2.9	4.3	4	480	280

* Per cent in 45 minutes.
† Grams per 100 ml.
‡ Patient 1 was studied by Lt. Col. Wm. Meroney, Dr. David Seligson, and Dr. V. M. Sborov, and the data are published with their permission.
§ Splenorenal shunts.

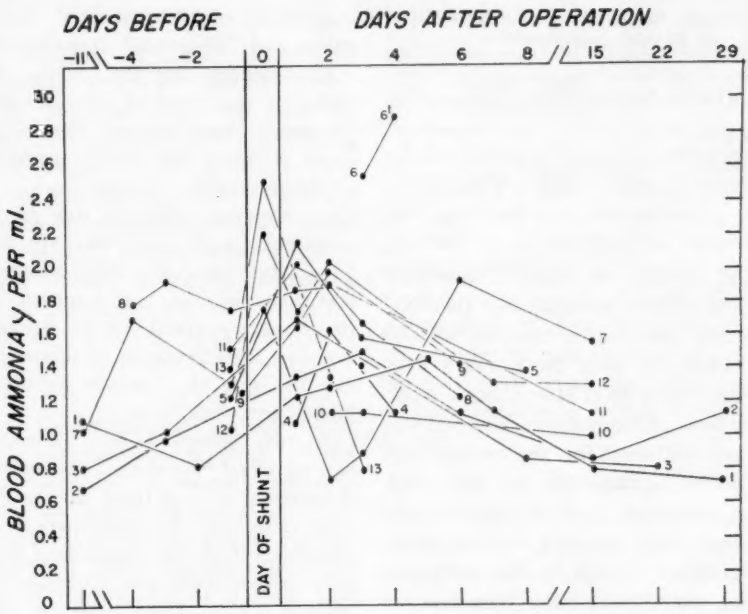

Fig. 1.—Effects of portacaval shunt upon the blood "ammonia."

more episodes of upper gastrointestinal hemorrhage, and in six the operation was a prophylactic one in patients whose varices had persisted in spite of conservative management. An end-to-side portacaval shunt was performed in 11 of the patients. Patient 4, with cirrhosis, had a splenorenal shunt because at laparotomy he was thought to be bleeding from varices rather than from a preoperatively diagnosed ulcer. A partial gastrectomy was necessary 14 days later when the ulcer continued to bleed. Patient 9 had a splenorenal shunt because no portal vein was available for anastomosis. Patient 6 died in coma on the fourth postoperative day. The rest were relatively well three months after operation, and all but Patients 4 and 9 (splenorenal shunts) had lost their varices by x-ray and esophagoscopy.

All patients received chortetracycline (Aureomycin), 500 mg. intravenously, for 48 to 72 hours after operation. By the seventh postoperative day the 12 patients who survived were out of bed and eating a normal diet.

Methods

Venous blood was drawn in the fasting state, and the concentration of "ammonia" was determined in triplicate by the method of Seligson.[8] The average standard deviation of the triplicates was 0.13μg. per milliliter. By this method, the upper limit of normal was 1.2μg. per milliliter.

TABLE 2.—*Mean Total Serum Bilirubin and "Ammonia" Concentrations Before and After Shunts*

Patient	Preoperative Controls		Postoperative Period			
			Days 1–6		Days 7–15	
	TSB *	"Ammonia" †	TSB *	"Ammonia" †	TSB *	"Ammonia" †
1	0.90	0.97	3.4	1.33	1.4	0.98
2	2.1	0.88	5.9	1.33	1.0	0.87
3	1.2	0.94	5.0	1.69	6.0	0.84
4	1.0	--	1.0	1.31	1.1	1.00
5	2.2	1.12	3.6	1.86	3.2	1.38
6	0.6	--	5.6	2.66	6.3	--
7	0.5	1.34	0.9	1.19	0.7	1.57
8	0.5	1.83	1.9	1.80	1.3	1.16
9	0.9	1.20	2.0	1.82	1.1	1.42
10	0.6	0.98	2.5	1.12	1.9	1.00
11	3.5	1.53	7.7	2.01	7.5	1.13
12	1.1	1.04	4.0	1.47	3.4	1.49
13	--	1.30	--	1.07	--	--
	1.26	1.19	3.79	1.59	2.91	1.17

* Micrograms per milliliter.
† Milligrams per 100 ml.

Results

The effects of the operation on each patient's blood "ammonia" concentration are illustrated in Figure 1, and in Table 2 are presented the mean blood "ammonia" and total serum bilirubin values for the week before and each of two weeks after operation. The Figure reveals that 8 of 11 pa-

TABLE 3.—*Mean Changes in Blood "Ammonia" and Statistical Significance*

	Patients, No.	Mean Change, μg./Ml.	Standard Error of Mean	P
Preoperative to first postoperative period	11	+0.40	0.11	<0.01
First to second postoperative period	11	−0.42	0.10	<0.01
Preoperative to second	10	−0.02	0.10	--

tients had a rise in blood "ammonia" immediately after operation, and the level was probably elevated in the two without control values. In most there was a drop to the preoperative level after about a week. Thus, the over-all mean for each period was 1.19μg. per milliliter for the preoperative control period, 1.59μg. for the first week after the operation, and 1.17μg. for the second week. The rise and fall are statistically significant (Table 3), but the values during the second week after operation are no different from the preoperative levels.

All the patients had a rise in total serum bilirubin immediately after operation, varying from 0.1 to 4.2 mg. per 100 ml. There was no significant correlation between the magnitudes of the individual rises in bilirubin and "ammonia" concentration.

Electroencephalograms in two of the patients at the time of the elevated blood "ammonia" were normal. One of these patients (Case 3) did develop marked electroencephalographic abnormalities a month after operation, when he was given ammonium chloride, 8 gm. a day, for four days. Five days later his electroencephalogram was again normal, and a month after this the syndrome could not be reproduced on repeated administration of ammonium chloride, in spite of a definite elevation of the blood "ammonia" level.

In two patients (Cases 12 and 13) the venous blood "ammonia" concentration was determined several times during the operation (Fig. 2). In both there was a rise associated with the operation, and in one there was a sharp rise after the shunt was opened, with a return almost to the preoperative level by the time the skin was sutured. In the other patient (Case 13) there was a steady rise throughout the operation, with a drop at its end, and opening the shunt had no additional effect.

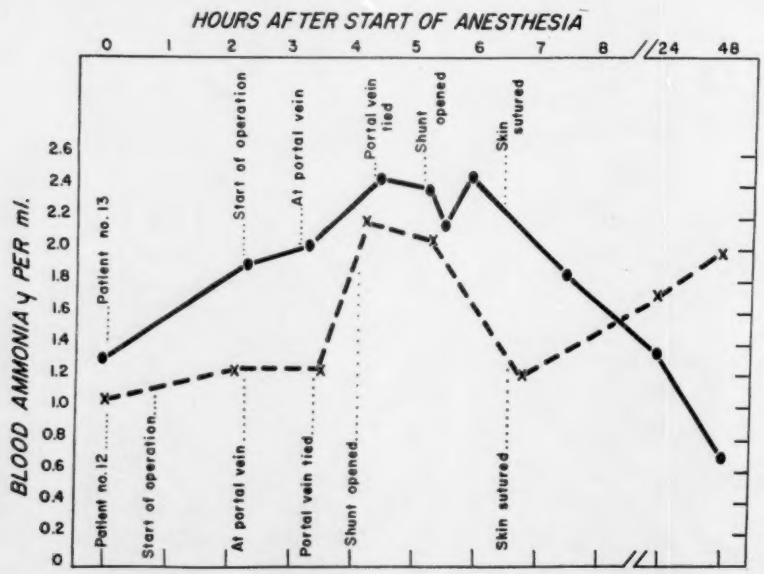

Fig. 2.—Immediate effects of operation and shunt upon the blood "ammonia."

Comment

The information most important to the interpretation of this study would be the effect of any abdominal operation upon the blood "ammonia" in patients with cirrhosis. It was not possible to obtain suitable data in a sufficient number of patients. Patient 4 had an emergency gastrectomy two weeks after his portacaval shunt without a rise in "ammonia." Patient 8 had an emergency umbilical herniorrhaphy a month before his shunt, without a rise, but he had practically no increase in "ammonia" following his shunt.

It is of interest that the average blood "ammonia" concentration returned to the preoperative level by the second postoperative week. At this time, all the 12 surviving patients were again eating a diet of normal protein content. This suggests either that the elevation during the first week was merely a reflection of postoperative liver functional impairment or that the return to normal in the second week is the result of some adjustment to the increased shunting of "ammonia" around the liver. It is impossible at this time to say which is the more important factor, but it should be pointed out that in cirrhosis with varices a great deal of blood is being shunted around the liver before operation, and the principal effect of the procedure may be to substitute a safe for an unsafe shunt. Previous studies[9,10] have shown that the blood flow through the liver is decreased by a portacaval shunt, but the decrease is not a striking or consistent one.

Three of these patients were studied by the metabolic balance technique, two on a high-protein diet (180 gm.), before and several months after the operation. Details of these studies are the subject of another paper.[11] No significant differences in the handling of protein in the pre- and postoperative periods were found, but in one of the three patients the average blood "ammonia" concentration was significantly higher during the postoperative study period.

It should be pointed out that all of the "ammonia" determinations in these studies were performed on blood obtained during the fasting state. Significantly higher blood "ammonia" values have been found during ammonium-citrate-tolerance tests following portacaval shunts,[12] and the levels are probably higher after a protein meal. It is still probable, however, that the occurrence of mental symptoms attributable to protein toxicity after portacaval shunt operations is not necessarily a bad effect of the operation but may rather indicate that the patient who has been saved from one complication of a progressive disease is now suffering from another.

Summary

The effects of portacaval shunt operations on the blood "ammonia" concentration were studied in 13 patients.

There was a rise after the operation which lasted a few days, but by the second postoperative week the "ammonia" concentration had returned to its preoperative levels.

Measurements during the operation in two patients suggested that opening the shunt had a significant effect on the blood "ammonia" level in one patient and added nothing to the "ammonia"-raising effects of the operation in the other.

It is suggested that the postoperative rise may be largely a result of liver-cell dysfunction accompanying the operation.

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An Analysis of Five Hundred Percutaneous Renal Biopsies

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No matter how important are new discoveries and methods, they cannot be considered to have reached fulfilment until generally applied to the sick.

W. B. Castle, 1939 *

The development of a safe [7] and relatively simple method of doing percutaneous renal biopsies has provided the physician and the clinical investigator with a new and useful tool for the study of renal diseases.[7,8] Elsewhere we have discussed in some detail its value to the patient and the physician.[9] By its use, exact histologic diagnoses can be made which provide a sound background for etiology, diagnosis, treatment, and prognosis. The procedure has also been used to obtain cultures of organisms from infected kid-

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* Dr. William Bosworth Castle spent part of August, 1938, in Melbourne, Australia, where he was the guest lecturer at a Refresher Course for Medical Practitioners, arranged by the Melbourne Permanent Postgraduate Committee. During the return trip to Boston, he and Mrs. Castle broke their journey at Soerabaja, Java, to make a pilgrimage to Eijkman's laboratory, at Weltevreden. That fall and winter, Dr. Castle described details of the pilgrimage and spoke in the Thorndike Laboratory and on the Wards of the Boston City Hospital of Eijkman's scientific observations on the etiology of beriberi—which later won him a Nobel Prize. These discussions were of more than passing interest to one of us (RMK), who had just started to work at the Thorndike. At that time the diagnosis and treatment of beriberi associated with alcoholism and pregnancy was under intensive study there by Drs. George R. Minot, Maurice B. Strauss, Soma Weiss, Robert Wilkins, and Arnold P. Meiklejohn.[2-6] Dr. Castle felt that Eijkman's scientific researches were doubly valuable. He had not only discovered a new physiologic principle, but his experiments on animals had led to developments which were of striking value to medicine and the patient.[1] Dr. Castle contended that the essence of clinical investigation was to discover new physiologic principles in man which could be directly applied to the prevention and treatment of disease. The fruit of the Castles' journey around the world and to and from Australia was a slim book in which these sentiments were expressed. Dr. Castle's aphorism which introduces our paper, written to honor him on his 60th birthday, was taken from the preface to his "Anemias and Vitamin Deficiencies," published obscurely in Australia, in 1939. It is a pity that this small book is not widely known, because it gives the reader a clear view of Dr. Castle's interest in the patient, in blood and food, and in the physiologic principles which govern their interaction in the body.

neys; to study, by serial biopsies, the natural history of diseases involving the kidney; to study reversible diseases of the organ, and to assess the effects of drugs on renal and cardiovascular diseases.[10-21]

Renal biopsies have also been used to investigate renal cytology and to study the morphology of the kidney through nephron dissection [18] or by electronmicroscopy.[22,23] In association with Sjoerd L. Bonting [24] the ultramicrobiochemical techniques developed by Lowry [25-27] have been adapted to study quantitatively the chemistry and the activity of various enzymes in different functional units of human nephrons taken by renal biopsy.

Since 1923, when Gwyn [28] described a case of glomerulonephritis diagnosed by biopsy of the kidney done during an abdominal operation, many physicians and surgeons have used an open operation to obtain renal tissue for diagnostic purposes or for clinical investigation.[29-39] In 1934, Ball [40] first used percutaneous aspiration biopsy to diagnose intra-abdominal masses, and he described a case of hypernephroma diagnosed by renal biopsy. In the next 16 years very few percutaneous biopsies were reported,[41,42] as most clinicians apparently agreed with Alwall [43] who—having done a dozen or so biopsies of the kidney—thought the procedure too dangerous to do. However, in 1950, Perez,[44] and in 1951, Iversen and Brun,[45] showed that in the right hands and with proper technique percutaneous renal biopsy was a safe clinical method. Iversen and his colleagues [46-57] were the first to make a systematic study of renal disease by percutaneous biopsy, and their work has led to the present interest in renal biopsy as a clinical and research tool.[58-79] In 1950, in Copenhagen, Dr. Iversen demonstrated his aspiration technique to us, but, as has been explained elsewhere,[7,8] we found that Iversen's method was difficult and, therefore, developed a new technique of percutaneous renal biopsy in the prone position which was efficient in extracting renal tissue and was relatively painless. The method we use has been adopted by Iversen and his colleagues for their studies [81] and it is now generally used to study patients in the United States [81-85] and abroad.[86,87]

An analysis of the first 100 cases was published in 1954 and 1955.[7,8] In those papers we discussed in detail the technique of percutaneous biopsy of the kidney in the prone position, as well as the indications for and contraindications to doing biopsies. Preoperative and postoperative measures taken to protect the patient, the management of complications, and the method of handling tissues and cultures were described. Modifications necessary in doing biopsies during operations, in pregnant women, or in patients who could not lie prone (by reason of disease or infirmity) were also described. It was established that the small amount of tissue removed from the kidney gave a representative picture of any diffuse parenchymatous renal disease that might have been present in the organ. In the present paper we present an analysis of the first 500 renal biopsies done by us (RMK, RCM, and VEP) or under our close supervision.†

Tools and Technique

The inherent difficulty with renal biopsy is in finding the organ with the biopsy needle. As described previously, we use intravenous pyelograms and an atraumatic fine exploring needle to locate the lower pole of the kidney before we insert the Franklin-Silverman biopsy needle (Fig. 1). In the prone position a sandbag placed under the abdomen pushes the kidney near the surface of the back and, through pressure, insures hemostasis after the biopsy specimen has been taken. The point of the fine exploring needle is in the kidney when that portion of it outside the skin swings through an arc with deep respiration.

No major modifications have been made of the previously described method. Our experience, however, has led us to emphasize certain aspects

† Since 1954, nine of our research fellows and associates have been taught to do renal biopsies on our patients, and we have also instructed twenty-six physicians and surgeons from other hospitals to use this method. Biopsies done during instruction and by our research fellows and associates are included in this report.

Fig. 1.—A drawing of the modified Vim-Silverman needle (now known as the Franklin-Silverman needle) which is used to take renal biopsies. Note in the insert the magnified view of the prongs, which are sealed at the beveled end of the groove.

of technique which have been useful to us. As these matters are of no particular moment to the general reader, they have been described in an appendix printed at the end of this paper, where they may be read by those interested in the technical details of renal biopsy.

Selection of Patients—Indications and Contraindications to Renal Biopsies

The indications for doing renal biopsies remain broad. As accurate and effective treatment can only follow on exact diagnosis, we are willing to do biopsies in any patient with diffuse renal disease who is able to undergo the procedure without danger and in whom a clear diagnosis has not been established.

At present we have not modified our position with regard to the contraindications to renal biopsy. These are listed in Table 1. Lich [85] believes that biopsies should not be done in patients with increased venous pressure due to congestive heart failure; but we do not believe that this is a serious contraindication to the procedure, as we have done a number of biopsies in patients with high renal vein pressure, without ill effects.

Iversen and his colleagues believe that biopsies can be done in uremic patients without hazard.[88] For example, they have done approximately 200 biopsies in patients ill with acute renal failure, and although many of these patients had a rising nonprotein nitrogen value, no untoward events ocurred. Although we have not as yet modified our position on the basis of Iversen and Brun's experience, we have done renal biopsies on patients with very high N. P. N. levels in the blood—as high as 259 mg/100 ml. None of these patients had a hemorrhagic diathesis; they were not uremic; their blood N. P. N. levels were stable; they had an adequate urine output. No complications occurred. Although we have not done biopsies in patients with uremia, we have seen in consultation two patients with chronic renal disease who were uremic and who had a rising N. P. N. The biopsies had been done by others, and the patients had bled following the biopsy. One patient had gross hematuria on the day of biopsy, and the second developed a hemorrhagic tendency five days after the biopsy. He had gross hematuria at home, became anemic, and needed blood transfusions, which controlled the hemorrhagic tendency. He made an uneventful recovery.

With regard to hypertension, it is proper to mention at this point that patients with severe hypertension developed complications more frequently than those with a normal blood pressure. In patients ill with malignant hypertension the biopsy should be done with great care and only when indications clearly warrant the procedure. More-

TABLE 1.—*Contraindications to Renal Biopsy*

1. Uncooperative patients
2. Large cysts
3. Renal neoplasms
4. Renal artery aneurysms
5. Marked calcific arteriosclerosis
6. Hemorrhagic diathesis
7. Single kidney
8. Perinephric abscess
9. Hydro- or pyonephrosis
10. Terminal state of illness
11. A blood nonprotein nitrogen level which is rising and which is over 100 mg./100 ml. of blood.

TABLE 2.—*An Analysis of the Diagnostic Adequacy or Inadequacy of Renal Tissue Taken by Biopsy*

	No.	%
Biopsies	500	100
Patients	368	
Biopsies repeated	132	
Renal tissue obtained	465	93
Tissue adequate for pathologic diagnosis	401	80
Tissue inadequate for diagnosis	64	13
No tissue obtained	35 *	7

* One piece of tissue lost in processing.

over, we believe that biopsies should not be done on patients with malignant hypertension until the operator has acquired considerable experience with the technique.

Limitations of Renal Biopsy

Even with the most careful technique it is not possible to obtain a satisfactory piece of renal tissue each time one attempts a renal biopsy. In Table 2 is recorded an analysis of the adequacy of the renal tissue obtained by biopsy. Although we obtained a piece of tissue from 93 out of every 100 patients we studied, the tissue was adequate for pathological diagnosis in 80% of the biopsies.

Renal tissue was considered adequate for pathological evaluation when five or more glomeruli were seen in the section. By studying serial sections, the maximum number of glomeruli can be obtained, thereby increasing the accuracy of the pathologic interpretation. The number of glomeruli seen in sections from each piece of renal tissue taken in the present series is shown in Figure 2. Of course, there are times when one can make a clear-cut diagnosis with less than five glomeruli per section, and we have done this on a number of occasions, such as when amyloid disease or chronic glomerulonephritis was present.

With regard to the failures, some occurred when we tried to obtain tissue from patients who were not completely cooperative and in whom we had been urged "to try to do a biopsy," because the diagnosis was in doubt. In others we had difficulties because of associated illnesses or deformities. Among such patients were those who had toxemia of pregnancy, ascites, rheumatoid arthritis, spondylitis, and other diseases involving the spine. Difficulties in obtaining adequate biopsies were also encountered in very thin patients (who had recently lost a great deal of weight), due to the extreme mobility of the kidney; in very obese or muscular patients, and in a high proportion of patients ill with malignant hypertension.

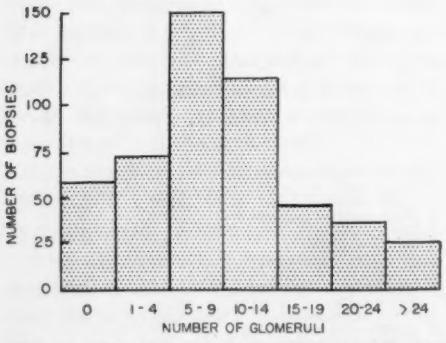

Fig. 2.—Distribution and numbers of glomeruli in sections of kidney tissue taken by percutaneous renal biopsy.

Complications and Symptoms Resulting from Renal Biopsy

It is most important to prevent complications, and careful attention should be paid to the pre- and postbiopsy studies and care, which are explained in detail in previous papers. The dangers of biopsy include laceration of the kidney or other organs, uncontrollable hemorrhage, and dissemination of infection. To these serious complications there may be added others, such as renal colic and back pain, which disturb the

TABLE 3.—*Analysis of Symptoms or Complications Which Appeared During or Following Forty-Nine Percutaneous Renal Biopsies* *

Complication or Symptom	No. Biopsies	% of 500 Biopsies
Deaths	0	0.0
Nephrectomy or operation	0	0.0
Anuria	0	0.0
Renal infection or bacteremia	1	0.2
Gross hematuria	26	5.2
Prolonged hematuria	3	0.6
Renal colic	14	2.8
Perirenal hematoma	3	0.6
Back pain	22	4.4
Mild ileus	2	0.4
Pain during biopsy	22	4.4
Transfusions	2	0.4

* A number of patients had two or more of the above complications or symptoms.

TABLE 4.—*Symptoms or Complications Which Appeared During or Following 500 Percutaneous Renal Biopsies*

Symptoms or Complications	No. Biopsies	% of Biopsies
None	418	83.6
Slight	33	6.6
Definite	49	9.8

patient. The symptoms and complications we have observed are listed in Tables 3 and 4. From these tables it can be seen that there were no deaths or other serious complications. The main problems have been pain and mild to moderate hemorrhagic incidents. Apart from the few who had pain during the biopsy, in most patients signs and symptoms of complications occurred within the first 30 to 60 minutes after the procedure was completed.

In most hospitals renal biopsies are done by interested and properly trained physicians, rather than surgeons, because, in general, renal biopsies are of little value in the diagnosis of surgical diseases of the kidney and are used to study the diffuse or so-called medical diseases of the organ. Therefore, it is necessary to ensure a close liaison and harmony between the physicians who do the biopsies and the urologic surgeons who may have to deal with the complications of the biopsy. Many of our patients were seen in consultation with the urologic service before the biopsies were done, and all patients who developed severe complications were seen by one of us (JHK) as soon as it became apparent that they had prolonged hematuria or severe pain.

Gross hematuria was seen 26 times and caused concern only when the patients developed renal colic or bled profusely. Gross hematuria usually stopped spontaneously within 6 to 12 hours, and we have only once had to give a transfusion to a patient because of massive hematuria. Gross hematuria has produced blood clots in the ureter or renal pelvis, and this is the cause of renal colic. The patient's fluid intake was increased as soon as we observed that the urine was heavily stained with blood, and we usually infused a liter or two of dextrose solution to induce a water diuresis and to try to prevent clotting. Renal colic following biopsy was seen in 14 patients. It was never prolonged, nor has it been of much moment. The pain was satisfactorily controlled with injections of meperidine (Demerol). However, the development of bacteremia, of prolonged hematuria, and of perirenal hematoma are disturbing and will be discussed in detail below.

Bacteremia.—This was seen in only one patient, and her history is described below.

CASE 1.—At the age of 9 years this patient had had acute glomerulonephritis followed by the nephrotic syndrome. When she was 18 years old, she entered hospital in the fifth month of her first pregnancy because edema of the legs had appeared. She had hypertension and azotemia, and protein and leukocytes were present in the urine, from which Aerobacter aerogenes and enterococci were cultured.

On March 11, 1955, a right percutaneous renal biopsy was done, apparently without incident. The following day, however, she had a fever for the first time—temperature to 102.6 F. Apart from slight tenderness at the biopsy site, the physical findings were unchanged. A. aerogenes was grown from the blood obtained from the kidney at biopsy, from the blood culture taken the following day, and on many occasions from the urine. The organism was found to be sensitive to chloramphenicol, and the patient was treated with this antibiotic in a daily dose of 4 gm. In addition she was kept at rest in bed and was given a daily fluid intake by mouth of not less than 3 liters. The fever subsided by the next day, but A. aerogenes was repeatedly cultured from the urine. The diagnosis of chronic glomerulonephritis was confirmed by the biopsy; in addition there was evidence of superimposed acute pyelonephritis.

Because of the acute pyelonephritis, termination of the pregnancy was advised, but this was not done because of a religious conflict. Under these circumstances it proved impossible to sterilize the urine, despite long-continued and vigorous antibiotic and sulfonamide therapy. The patient died on June 3, 1955, in the eighth month of her pregnancy, from severe acute pyelonephritis with multiple microabscesses throughout both kidneys.

It is apparent from the above that dissemination of infection to the blood stream can occur if the kidney is infected. However, it is a most uncommon complication. Jackson and his colleagues have biopsied the kidneys of 220 patients ill with pyelonephri-

tis and have noted that bacteremia developed in only 2 patients.[89] In each instance the bacteremia occurred some hours after the biopsy, and high fever and chills were associated with shock due to the presence of Gram-negative coliform organisms in the blood stream.

Prolonged Hematuria.—This was observed in three patients. It is our custom to discharge patients from the hospital 24 hours after the biopsy is done. At this time we advise them to report to us if they notice hematuria, pain, fever, chills, or other untoward happenings. Three patients returned to us, complaining of recurrent hematuria following their return to their homes. Each time they took to their beds, the hematuria cleared, but when they were up and about, bloody urine was noticed.

The following case history illustrates the problems of management of prolonged hematuria.

CASE 2.—A 36-year-old frail Negro mother had the nephrotic syndrome due to severe chronic glomerulonephritis. Two days after a renal biopsy was done she developed gross hematuria.

Prior to renal biopsy her blood pressure was 150/90; the hematocrit was 35%, and the blood nonprotein nitrogen was normal. However, tests of renal function were found to be slightly impaired. Renal biopsy was done on Oct. 11, 1954, without any immediate sequelae. Her urine was grossly clear of blood, and she was discharged the next day. On Oct. 13, 1954, she passed smoky-colored urine. The smoky appearance cleared considerably, but not completely. By Oct. 18 the urine was a deep red color. Although she had no associated fever, pain, or dysuria, she was admitted to hospital. Her temperature was normal, and her blood pressure was 160/90. There was no kidney tenderness. The hematocrit was 33%, and the urine was grossly bloody. She was kept in bed and given large quantities of fluids to drink. Repeated culture of the urine revealed no bacterial growth. By the third hospital day the hematuria subsided. After five additional days in bed she was allowed to get up. During the next two days neither microscopic nor gross hematuria appeared, and she was discharged. One week later gross hematuria recurred and was associated with the passage of several blood clots and mild back pain. She was readmitted to the hospital. Her temperature was normal. The blood pressure was 160/94. The hematocrit and blood nonprotein nitrogen remained unchanged. The clotting, bleeding, and prothrombin times were all normal. The urological surgeon (JHK) was consulted. He suggested retrograde pyelograms. Slight calyceal ectasia was noted, and a kink of the right ureter was seen just distal to the ureteropelvic junction. This was associated with mild hydronephrosis and hydroureter. Anerobic streptococci grew from a sample of urine taken by catheter. A regimen to promote healing was instituted. She was kept flat in bed for 10 days. The fluid intake was increased to produce over 2 liters of urine each day, and the urinary infection was treated with penicillin and sulfisoxazole (Gantrisin). Erythrocytes disappeared from the urinary sediment, and urine cultures became free of bacterial growth. She was discharged on Nov. 29, 1954, and had no recurrence of hematuria.

When the patient has prolonged hematuria, it is important to give the wound in the kidney a chance to heal. To prevent movement of the kidney, the patient should be kept flat in bed for 10 days. The foot of the bed should be raised 18 in. to keep the ureter as straight as possible. The patient should be encouraged to drink up to 4 liters of fluid each day, and intravenous infusion of dextrose should be given if this cannot be done. A broad-spectrum antibiotic is prescribed to prevent renal infection. These patients should also be seen in consultation with the urologist, and he should decide on additional investigations, such as retrograde pyelography. Naturally, tourniquet tests, studies of platelets and clotting, bleeding, and prothrombin times should be done. The urine should be cultured, and a blood culture should be taken if there is fever.

Perirenal Hematoma.—This is the most serious and troublesome complication we have observed. Bleeding occurs in the perirenal fat, and relatively small amounts of blood accumulating in this area may produce pain, shock, and a fall in hematocrit value quite out of proportion to the amount of blood which has extravisated. Pain is severe, continuous, and prolonged and is not very well controlled with the usual amounts of narcotics. Usually the first symptom is pain in the back, followed by nausea and vomiting. Spasm and guarding of the back muscles may be found on examination, and

later a mass can usually be found by bimanual palpation of the abdomen. The extent of hemorrhage in the back can best be evaluated by serial tomographic x-ray studies. Pain and guarding may last for two to three days and then gradually subside. It is most important that the urologic surgeon be called to see these patients as soon as the diagnosis of perirenal hemorrhage is suspected. It is the urologist's decision whether to adopt conservative management or whether to operate, and it is only fair to have him in the picture as early as possible. The following case history illustrates the development of perirenal hematoma in one of the three patients who developed this conplication.

CASE 3.—A 22-year-old Negro woman had the nephrotic syndrome due to chronic glomerulonephritis. She was closely followed by serial tests of renal function and serial renal biopsy. After the third renal biopsy, she developed a perirenal hematoma. Prior to the third renal biopsy she was seriously and chronically ill. Her blood pressure was 180/100, and the blood nonprotein nitrogen was 66 mg/100 ml. The hematocrit was 23%. She was hospitalized and given three 250 ml. transfusions of packed erythrocytes. Following this, her hematocrit rose to 38%.

On March 7, 1955, immediately following a third renal biopsy, she complained of severe pain in her right flank as well as in the right upper abdomen. While still lying prone on the sandbag, marked spasm and guarding of the right long lumbar muscles appeared. The pulse became rapid, and the blood pressure fell slightly. The intense right loin pain was relieved by pressure to the biopsy site. Her pulse rate returned to normal, and the blood pressure rose to prebiopsy levels.

Twenty minutes after the kidney biopsy was done, the right loin pain had greatly subsided, but not completely. Immediately upon being placed flat on her back in bed she again experienced right loin pain. Now spasm was noted of the right rectus muscle, and a soft mass approximately 3 to 4 in. in diameter was palpated anteriorly in the region of the kidney. Repeated injections of meperidine were given to relieve flank pain. Three liters of dextrose solution was given to produce a diuresis. This was given to avert any possible renal colic. Bowel sounds were present, and rebound tenderness was absent. The next morning the patient was more comfortable, with less pain in the right kidney region. Her hematocrit was 34%, and 250 ml. of packed erythrocytes was slowly infused. Over the next two weeks she gradually improved and was well enough to be discharged.

On March 19 she was ready to go home, when she suddenly became dyspneic as a result of acute pulmonary edema. Over the next three weeks her course was downhill, and she died in uremia, with pulmonary edema, electrolyte imbalance, and cerebral encephalopathy.

Postmortem examination revealed that the right kidney was surrounded by an organized pericapsular hematoma (approximately 250 cc. in volume). The hematoma weighed 200 gm. Each kidney weighed 140 gm. The right psoas muscle and connective tissue adjacent to the hematoma were indurated and fibrotic. The Pathologist attributed death to electrolyte imbalance and pulmonary edema superimposed upon advanced glomerulonephritis.

It can be seen from Table 4 that 22 patients had some back pain on the day of the biopsy which was readily controlled by simple measures—usually bed rest and injection of meperidine. It is quite possible that in a number of instances this pain may have been due to a small perirenal hematoma. Two patients also developed abdominal distention and a decrease in bowel sounds on the day of the biopsy. There was no evidence of fever, shock, or other untoward symptoms or signs, nor was there any suggestion that the bowel had been punctured by the needle. Presumably these were cases of mild reflex ileus, and the distention passed off rapidly without any special treatment. Twenty-two patients also had some pain during the biopsy procedure which was not always due to inadequate local anesthesia. In one patient, pain came on suddenly as the biopsy specimen was taken. A portion of the myocalix muscle was seen in the section of the tissue from this patient.

The Clinical Value of Renal Biopsy

Nineteen years ago Iversen and Roholm [90] introduced a safe technique for percutaneous needle biopsy of the liver. This was followed by its widespread use in the diagnosis, management, and investigation of diseases affecting that organ. Since then, the clinical and histologic picture of the most important diseases of the organ have been delineated, and the results which have been achieved

by hepatic biopsy are apparent to all. The value of percutaneous renal biopsies to the patient are no less than for the liver, and its potentialities are beginning to unfold.

With regard to diagnosis, the histologic diagnosis is useful because knowing exactly what is wrong with the patient's kidney provides a more rational approach to therapy and also stimulates the physician to continue to seek the latest therapeutic advances in the treatment of the particular disease he has uncovered. Parrish and Howe [60] found that renal biopsy established the diagnosis in 52% of patients studied when the clinical impression was incorrect; it confirmed the clinical impression in 39% of studies, and in 9% neither the pathologist nor the clinician could arrive at a definite diagnosis. Kark and Muehrcke [7] published similar figures. Renal biopsy has been particularly useful in the diagnosis of unsuspected inflammatory disease of the kidneys, in determining exactly the organism responsible for infection, and in the diagnosis of systemic lupus erythematosus and other diseases of the ground substance and connective tissues (so-called collagen diseases). It is especially of value in the diagnosis of the nephrotic syndrome, which has been shown to be associated with a wide variety of pathologic patterns.[91] In many instances of this syndrome a careful history, a thorough physical examination, and laboratory investigations—including tests of renal function—did not help in making a differential diagnosis. If azotemia was severe and if renal function was markedly impaired, then one could usually predict that there was severe structural damage to the kidney. The reverse did not hold: there were seen on occasion marked structural changes in the glomeruli with little alteration in renal function, and with comparatively normal glomeruli there were found mild or moderate or severe degrees of azotemia. It was these circumstances that made renal biopsy so valuable a tool in the exact diagnosis of the nephrotic syndrome.

Renal biopsy is also of value in the treatment of renal disease. Brun [5] has used renal biopsy to select anuric patients for dialysis on the artificial kidney. He found that patients whose glomeruli were severely damaged did poorly, and those with acute lesions responded best. Bjørneboe and his colleagues [48] have used renal biopsy to select patients with the nephrotic syndrome for treatment with corticotropin (ACTH) or steroid hormones, and we have confirmed their results.[91] The diuretic response to hormone therapy appeared only in patients with minimal histologic evidence of glomerular damage. Renal biopsy also appears to be a more accurate method than culture of the urine in determining exactly the organism responsible for infection within the kidney.[9,83]

In conclusion, we are convinced that each biopsy specimen we examine profoundly affects the management of the illness,[9] while the tissue harvested by percutaneous biopsy provides the most significant evidence on which to forecast the course of a disease involving the kidneys.

Summary

An analysis of symptoms and complications which were observed during or following 500 percutaneous renal biopsies done in 368 patients is reported. Definite symptoms or signs of complications were observed in 9.8% of 500 consecutive renal biopsies. There were no deaths, and no patient required an operation. Anuria was not observed. The only serious complication was perirenal hematoma, which was seen in three patients and which was treated conservatively. Fourteen patients developed renal colic, and three had prolonged hematuria. These two complications were readily controlled with simple measures. Renal tissue was obtained in 93% of all the biopsies; the tissue was adequate for pathologic diagnosis in 80% of the biopsies. The usefulness of the procedure far outweighs the morbidity, and one may now consider that percutaneous renal biopsy in the prone posi-

tion is a practical method which can be generally applied to the care of the sick.

Many colleagues at Presbyterian-St. Luke's Hospital, the Research and Educational Hospitals of the University of Illinois, Cook County Hospital, and elsewhere referred patients to us for study.

The house staff and nursing staff of these hospitals helped in the study.

Dr. Robert Lich, of Louisville, and *The Journal of the American Medical Association* gave permission to reproduce Figure 1.

University of Illinois College of Medicine, 1853 W. Polk St. (12).

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Appendix—Modifications in Technique of Renal Biopsy

1. *The Biopsy Needle.*—It is quite clear to us that the commonly used, unmodified Vim-Silverman needles does not extract renal tissue efficiently. We have received a number of letters from clinicians complaining that they were unable to obtain renal tissue even when they were sure that the biopsy needle was in the kidney. In investigating these complaints, it appeared that the clinicians were using the wrong needle—a standard Vim-Silverman needle. Their difficulties were solved when they changed

over to a properly tooled Franklin-Silverman needle (Fig. 1). A further complaint concerned the length of the original needle. It was too short to use on very obese or muscular patients. At present we use a 3¾ in. needle for most of our biopsies, but we also employ a short, 2½ in., needle for infants and children and on very rare occasions a long needle (5¾ in.) for very obese patients (less than 1%).

The original Franklin-Silverman needle is rather top-heavy, and the hub is made of steel. It may hang down the back if it is used when the biopsy is to be taken in the upright position or if the needle is used to take a biopsy in a very thin person or a small child. To overcome this difficulty, one of us (RCM) developed a steel needle made with an aluminum hub.† This is a well balanced instrument, and we prefer it to the steel-hubbed one.

2. *Technique.*—With regard to the technique of renal biopsy, the following additional points have been of value to us.

Finding the Exact Depth of the Kidney: A 6 in. 22 gauge exploring needle is advanced into the renal cortex. When the tip of this needle is in the kidney, the needle moves with respiration through a distinct arc in the long axis of the body, the hub moving with inspiration and with expiration. In most instances this arc of swing is definite and is easily recognized. However, the swing may be small in some and in others may not be clearly observed—especially if the patient is too nervous to breathe quietly and deeply. If this occurs, collateral evidence must be sought to enable the operator to localize the site and depth of the kidney.

(*a*) The experienced operator, who advances the exploring needle gradually and deliberately, learns to "feel" the structures through which the needle passes. After piercing the lumbodorsal fascia, the needle meets no resistance until it impinges upon the renal capsule. The needle is advanced slowly and deliberately at this point. As it enters the kidney a resistance is felt. This resistance may be barely perceptible when the edematous kidney of the nephrotic patient is punctured. By contrast the contracted fibrotic kidney may feel extremely firm or gritty.

(*b*) Occasionally a distinct arterial pulsation is seen at the hilt of the needle. This pulsation is transmitted from the vessels in the kidney and is seen only when the exploring needle is in the kidney; it is not seen when the tip of the needle is in perirenal fat.

(*c*) An additional movement of the exploring needle is seen occasionally. As the patient breathes, the needle may rotate around its long axis through an arc of about 5 to 15 degrees. This rotation occurs only when the needle is in the kidney.

(*d*) A sharp flick or sustained vibration of the needle may be seen during inspiration. It occurs when the tip of the needle lies immediately adjacent to, but not within, the renal capsule, i. e., when the needle is immediately below, medial, lateral, or posterior to the kidney but not within the kidney capsule. The sides of the kidney flick the needle during inspiration, while the vibration is caused by the tip of the needle scratching the dorsal surface of the kidney as it moves down with each breath. In either case the needle is very near the capsule of the kidney, and only a small manipulation is needed to push it into the cortex.

These four additional points have proved useful to us in localizing exactly the site and depth of the kidney. Although the movements described above are most clearly seen with the long, thin, exploring needle, they may also be observed when the Franklin-Silverman needle is introduced to take the biopsy.

3. *Infants and Children.*—No major modifications in technique are used to locate the kidney or take the biopsy specimen in infants or very small children.

† The Franklin-Silverman needles can be obtained from V. Mueller & Company, Chicago, or the Edwards Surgical Supply Company, London, England.

Children over the age of 7 or 8 will usually cooperate and do not need anesthesia. However, infants and very young children are deeply sedated with chloral hydrate, and the exploring and biopsy needles are introduced while the child holds its breath during crying spells. To date 19 biopsies have been made in children under 12 years of age. In our opinion, operators should not do percutaneous renal biopsies on infants and children until they have had considerable experience with adults.

4. *Biopsies with the Patient Sitting up.*—When the biopsy has to be done when the patient sits up, it is often impossible to obtain tissue with the Franklin-Silverman needle, as the organ is pushed forward by the needle or by the prongs as they are advanced to bite the tissue. This difficulty can be overcome by arranging for an assistant to push firmly against the front (that is, the ventral surface) of the patient's abdomen with both hands. He starts to do this a few seconds before the needle is pushed into the kidney, and the pressure is kept up until the biopsy specimen is secured.

5. *Contamination of Needle with Organisms.*—It is our custom to culture the renal biopsy needle, renal blood, or renal tissue after each biopsy has been done. Investigations by Dr. Charles Wolfe and Dr. Nicholas Menolasino, at Presbyterian Hospital, indicate that organisms from the operator's oral cavity and nose may contaminate the instruments and operative field. To insure sterility we now wear masks.

6. *Cooperation by the Patient.*—It is worth while stressing once again the need for full cooperation by the patient during the operation. This can be achieved by gentle and detailed explanation of the procedure the night before the operation and by making the patient practice deep breathing while lying in the prone position. It is our custom to tell the patient about the potential hazards of the procedure and to explain to him the measures taken if the hazards arise.

When the biopsy is under way, one of our team takes the patient's pulse and blood pressure. He also spends his time reassuring and encouraging the patients. His vocal anesthesia is particularly useful with nervous patients and children between the ages of 8 and 15. If he can entrance children with exciting and vivid stories while they are having the biopsy done, so much the better.

Physiologic and Pathologic Alterations Produced by the Endotoxins of Gram-Negative Bacteria

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The endotoxins of Gram-negative bacteria, sometimes referred to as the "bacterial pyrogens," are the macromolecular lipopolysaccharide complexes which comprise the somatic antigens of these bacteria. Although distinctly different from each other with respect to antigenicity, depending on differences between the polysaccharides of various species, they are remarkably similar in the physiologic and pathologic reactions which they produce in animals. According to Westphal,[1] the lipid component is responsible for toxicity and is common to all endotoxins. The complexity of the response to endotoxin is illustrated by a partial list of the mechanisms brought into play by a single injection: fever, leukopenia followed by leukocytosis, peripheral vasoconstriction and vasomotion, shock, depletion of liver glycogen, hyperglycemia, increased adhesiveness of polymorphonuclear leukocytes, heparin-precipitability of fibrinogen, impeded phagocytosis by reticuloendothelial cells, augmentation of antibody response to protein antigens, abortion, hemorrhagic necrosis in malignant tumors, and disturbances of the reactivity of terminal blood vessels to epinephrine.[2-4] The range of susceptible hosts includes a broad biological territory, extending from 10-day-old chick embryos to man. With two or more injections, the effects become even more complicated, with the appearance of such phenomenon as local and generalized Shwartzman reactions, tolerance, and induced resistance to bacterial infections and traumatic shock.[2]

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At the present time, there is no single primary action of endotoxin on any known tissue component which can be invoked to explain the numerous effects of endotoxin, and it is more likely that a mass of complicated and interlocking reactions, many of them secondary or tertiary, are involved. The sequence and timing of events seem as important as the events themselves in determining the final outcome.

As an approach to the problem, studies in this laboratory have been directed toward the sorting out of certain individual reactions in the hope of uncovering common underlying mechanisms. This paper is concerned with four aspects of endotoxin: (1) the role of the reticuloendothelial system in defense, (2) the dissociation between the primary lethal effect of endotoxin and certain other tissue-damaging properties, (3) the role of fibrinogen in the formation of intravascular fibrinoid in the generalized Shwartzman reaction, and (4) the role of epinephrine in the local and systemic necrotizing actions of endotoxin. This is not intended to serve as a comprehensive review of the numerous actions of endotoxin; for such, the reader is referred to other publications.[2,5,6]

The Reticuloendothelial System and Resistance to Endotoxin

When animals are given daily injections of sublethal amounts of endotoxin, they rapidly become resistant to the material, so that within 7 to 10 days they can be given doses which would normally be lethal without exhibiting any untoward symptoms. This state of affairs, usually referred to as tolerance, applies to all of the known reac-

tions of endotoxin,[2] including fever, leukopenia, the local and generalized Shwartzman reaction, hyperglycemia, depletion of liver glycogen, tumor necrosis, and the epinephrine lesions described below.

Tolerance is immunologically quite nonspecific. Animals with tolerance for one type of endotoxin are also resistant to other immunologically unrelated lipopolysaccharides. Moreover, the state is accompanied by a remarkable degree of enhanced resistance to infection by a wide variety of heterologous micro-organisms,[7,8] and there is also an increase in resistance to traumatic shock.[4,9,10]

There is reason to believe that tolerance, and probably also the natural resistance to exdotoxin of normal animals, may represent a function of the reticuloendothelial system. Beeson [12] showed that tolerance to the pyrogenic action of endotoxin could be eliminated almost immediately by an intravenous injection of colloidal thorium dioxide or trypan blue and suggested that this was due to "blockade" of the reticuloendothelial cells by these substances. Later, Good and his associates found that the resistance of normal animals was decreased more than one-thousand-fold by the same and other colloids (colloidal carbon and saccharate of iron oxide).[13,14] Oddly enough, it has been shown that the increased resistance to traumatic shock in endotoxin-tolerant animals is also eliminated by an injection of these colloidal materials.[9]

On the basis of these observations, it has been suggested that reticuloendothelial cells are somehow concerned with the removal or detoxification of endotoxin. The rapid disappearance of endotoxin from the circulating blood in normals and the increased rate of clearance encountered in tolerant animals are thought to be manifestations of different levels of activity on the part of these cells.[12]

It is, however, difficult to interpret observations made with such things as colloidal thorium dioxide or carbon without a more precise understanding of the action of these materials than is available at present. The basis for assuming that they change reactivity to endotoxin by virtue of their effect on reticuloendothelial cells is largely that they are rapidly and quite selectively taken up by these cells, but too little is known about other changes that may be occurring at the same time in constituents of the circulating blood or in other cellular elements of the vascular tree.

Some help in this matter has been provided by Benacerraf and his associates, who have devised a sensitive quantitative procedure for measuring the rate of uptake of colloidal carbon by the reticuloendothelial cells in mice, rats, and rabbits.[15] These investigators have reported that the rate of clearance is significantly impeded by a prior injection of colloidal material, indicating justification for the term reticuloendothelial "blockade." They also showed that endotoxin itself affects the functioning of reticuloendothelial cells [16] in a biphasic manner that may account for some of the observations described above. First, during a period of approximately 12 hours after injection there is a precipitous drop in the rate of carbon clearance from the blood. It is during this same time that the animal is most susceptible to the lethal effect of a second injection of endotoxin, according to Schweinburg et al.[17] After this, phagocytosis returns to normal and then, at about 24 hours, increases to a rate considerably above normal, coinciding with the period when acquired resistance to endotoxin is first exhibited.[18]

It might be hypothesized, then, that after an intravenous injection endotoxin is cleared from the blood by the reticuloendothelial cells and, as a result, the functioning of these cells is at first impaired and later stimulated. In the same terms, a prior injection of colloidal thorium dioxide (thorotrast) or other colloidal material may interfere with the clearing mechanism, so that an injection of endotoxin now finds the reticuloendothelial system unable to remove or detoxify, thus enabling endotoxin to pro-

duce damage to other vulnerable tissues.[13,19] This view, although it seems to fit with the available information, awaits actual proof; labeled endotoxin might be extremely useful here.

Pillemer has reported that variations in resistance to the lethal effect of endotoxin seem to be related to changes in the level of properdin in the blood and suggests that an interaction between endotoxin and properdin may be an important event in determining the systemic action of endotoxin.[20] Whether changes in properdin levels can be accounted for on the basis of changes in the activity of reticuloendothelial cells is not known.

Johnson and his associates have recently described the remarkable capacity of endotoxin to act as an adjuvant to immunization by various protein antigens.[21] When endotoxin is injected simultaneously with antigen, the titer of antibody subsequently formed is consistently much higher than in controls. It has been suggested that the effect represents some kind of action on the reticuloendothelial system, perhaps by altering the distribution or rate of uptake of the antigen by precursors of antibody-forming cells.

Dissociation of the Primary Lethal Effect from Other Actions of Endotoxin

It is now generally accepted, on the basis of reports from many laboratories, that the shock-producing lethal effects of a single large dose of endotoxin can be completely prevented by prior treatment with cortisone.[2,22,23] As seems the case with virtually all actions of cortisone, the mechanism underlying this one remains unknown. It has been observed in several mammalian species, including rats, mice, guinea pigs, rabbits, and man. It is also demonstrable in the 10-day chick embryo, in which a drop of endotoxin on the choriallantoic membrane results in generalized embryonal hemorrhage and death within a few hours; administration of cortisone in small doses just before endotoxin provides complete protection against this effect.[24]

However, protection against the primary lethal action of endotoxin does not mean that cortisone will protect against other actions. Indeed, it is evident that the mechanisms involved in shock and death after a single large dose of endotoxin are quite different from those mediating the Shwartzman reaction, and the two types of reaction can be dissociated from each other by experiments with cortisone. In the generalized Shwartzman reaction, cortisone may prevent the early symptoms of weakness and collapse after each dose of endotoxin, but it does not prevent the reaction itself.[23,25] In the local Shwartzman reaction, the extent of local skin inflammation at the prepared site is much diminished by cortisone, but the reaction of hemorrhagic necrosis which follows the intravenous "provoking" injection is not at all affected.[25] Indeed, the cortisone-treated rabbit behaves as though already prepared for both kinds of Shwartzman reaction: a single intradermal injection of endotoxin gives rise to multiple petechial hemorrhages in the skin 24 hours later, and a single intravenous injection is followed by fibrinoid deposition and the development of bilateral renal cortical necrosis.[25]

This lack of interdependence between the mechanisms of lethal shock and those of the Shwartzman reaction has been encountered in other pharmacologic studies. For example, the early lethal reaction to intravenous endotoxin in the rabbit is prevented by chlorpromazine, and, less uniformly, phenoxybenzamine (Dibenzyline), but chlorpromazine and phenoxybenzamine have no protective action against either the local or generalized Shwartzman reaction.[4] On the other hand, heparin and mechlorethamine hydrochloride (nitrogen mustard), which completely block both kinds of Shwartzman reaction, have no influence on the shock-producing lethal action of endotoxin.[2,26,27]

A similar dissociation of the reactions is to be seen in the different effects of endotoxin on rabbits of different ages. Young

sexually immature rabbits are extremely resistant to the shock-producing action, and many will survive doses of purified lipopolysaccharide as high as 500μg. In contrast, full-grown rabbits rapidly become prostrated and die in shock after receiving only 5μg. to 10μg.[28] The relative susceptibility of the two groups to the generalized Shwartzman reaction is quite the reverse; the reaction can be produced with regularity in young rabbits but is a rare event in mature animals. It is curious that age plays an equally important part in the susceptibility of chick embryos to endotoxin; 7- or 8-day embryos show little or no reaction, while at 10 days the majority are rapidly killed by small doses. Later, at 13 or 14 days, susceptibility again disappears, and newborn as well as mature chickens show no reaction to endotoxin.[24]

The Origin of Intravascular Fibrinoid in the Generalized Shwartzman Reaction

The generalized Shwartzman reaction is produced in rabbits by the intravenous administration of two sublethal doses of endotoxin, spaced approximately 24 hours apart. During the day following the second injection the kidneys undergo bilateral cortical necrosis; it is this characteristic lesion that identifies the generalized Shwartzman reaction as such.[27,29,30]

The mechanism responsible for renal cortical necrosis is the deposition within glomerular capillaries, occluding these vessels, of dense masses of homogeneous eosinophilic material with the staining properties of fibrinoid.[27,29] Similar masses are laid down within the sinusoids of the liver and spleen and beneath the endothelium of coronary arteries and mitral and aortic valves.[29,31] Electron microscopic examination of the glomerular fibrinoid shows it to be composed of unbranched fibrils exhibiting a periodicity of 120 A.[32]

The lesions of the generalized Shwartzman reaction are completely prevented by treatment with heparin at the time of the second injection in doses sufficient to keep the blood incoagulable for several hours.[26]

Because of the morphologic appearance of intravascular fibrinoid and the effect of heparin, an investigation of the role of the coagulation mechanism in the generalized Shwartzman reaction was undertaken. It was found that one of the consistent effects of an intravenous injection of endotoxin was a curious instability of blood fibrinogen, which developed during a period of two or three hours after each injection. Heparinized whole-blood samples tended to clot much more readily than in normals, even when large amounts of heparin were used, and became solidified within a few minutes after being placed in the refrigerator. It was then observed that the addition of heparin to chilled samples of citrated or oxalated plasma, obtained within one to four hours after an injection of endotoxin, resulted in the formation of heavy masses of flocculent precipitate. On warming, the precipitates usually went back into solution promptly (although if the tubes were kept for several hours in the cold, solid rubbery masses resembling fibrin remained after warming). The heparin-precipitable material was found to be composed in large part of fibrinogen, which had apparently undergone partial polymerization in the direction of fibrin and possessed an affinity for heparin which enabled it to be precipitated by this substance at low temperatures.[3,33,34]

While the study of heparin-precipitable fibrinogen was in progress, Walton[35] reported his observation that dextran sulfate, a heparin-like acidic polymer of large molecular size, caused flocculent precipitation of fibrinogen when added to normal plasma. Precipitation was prevented by the addition of heparin, in optimal proportions, and could be reversed by excess heparin, suggesting that competition was taking place between dextran sulfate and heparin for a combining site on fibrinogen.

Walton also found that intravenous injections of lethal doses of dextran sulfate into rabbits and guinea pigs resulted in

fibrinoid occlusion of the glomerular capillaries and bilateral cortical necrosis of the kidneys, with lesions indistinguishable from those of the generalized Shwartzman reaction.[36] Similar lesions were described by Hausmann and Dreyfus following injections of Liquoid (sodium polyanethol sulfonate), another heparin-like polymer of large molecular size.[37]

In view of these findings, together with the known presence of heparin-precipitable fibrinogen after endotoxin, the effect of injecting various doses of the heparin-like acid polymers into rabbits previously given endotoxin was studied. One hour after a single injection of endotoxin, at the time when fibrinogen was maximally precipitable by heparin, an intravenous injection of small doses of dextran sulfate caused rapidly fatal reactions, with massive intravascular deposits of fibrinoid and bilateral renal cortical necrosis.[38,39] Similar results were obtained when endotoxin was followed an hour later by Liquoid, or by sodium polyvinylalcohol sulfonate, employing doses of these materials which had no detectable effect when given alone. The synergistic effect of the acidic polymers with endotoxin was not demonstrable when the polymer was injected prior to endotoxin or when delayed until eight hours after endotoxin. Electron microscopy of the glomeruli showed the intravascular material to have the same fibrillar structure and periodicity as the fibrinoid in the conventional generalized Shwartzman reaction.[32]

As in the reactions produced by two doses of endotoxin, the polymer-endotoxin reactions were completely prevented by heparin when it was given just before the injection of polymer. Heparin-treated rabbits showed no evidences of intoxication and failed to develop either intravascular fibrinoid or renal necrosis.[38] It is possible that this protection represents successful competition by heparin for a site on fibrinogen, as is the case in its prevention of in vitro precipitation of fibrinogen by dextran sulfate.[35]

The probable participation of fibrinogen in the formation of intravascular fibrinoid was shown in the following experiment:

Three groups of rabbits were used. One received a single injection of Liquoid, the second, a single injection of endotoxin, and the third, an injection of endotoxin followed one hour later by Liquoid. Blood fibrinogen levels were determined just before and at intervals after the injections. No changes in fibrinogen occurred in the first two groups. In contrast, the animals receiving endotoxin followed by Liquoid showed abrupt depletion of fibrinogen, amounting often to complete disappearance of this material from the circulating blood, during the hour following the injection of Liquoid. All of these had dense masses of fibrinoid in the glomerular capillaries when killed at the end of the hour.[38,39]

Next, a fourth group was given endotoxin followed by Liquoid, but heparin was administered just before the Liquoid. None of these animals showed depletion of blood fibrinogen, and none developed intravascular fibrinoid or renal necrosis.[38]

It was thus evident that the acidic polymers were capable of imitating the action of one of the two injections of endotoxin in the generalized Shwartzman reaction, although the timing of injections was obviously quite different. It seemed likely that the alteration of fibrinogen to its heparin-precipitable form was implicated in its in vivo precipitation by Liquoid, dextran sulfate, or polyvinylalcohol sulfonate. The question was, therefore, raised as to the nature of the event which, after two doses of endotoxin, brought about fibrinogen precipitation. What component of the conventional generalized Shwartzman reaction was being mimicked by the acidic polymers?

A possible answer was provided by experiments with mechlorethamine hydrochloride.[38] It had previously been established that the generalized Shwartzman reaction is, like the local reaction, consistently prevented by mechlorethamine hydrochloride. The protective action appears to be directly correlated with the polymorphonuclear leukopenia caused by mechlorethamine hydrochloride, indicating that something of basic importance is contributed by the blood leukocytes in the generalized Shwartzman reaction.[27,40] In contrast, mechlorethamine

hydrochloride had no protective effect on the incidence or severity of the lesions produced by endotoxin in combination with Liquoid or dextran sulfate.[38]

It is possible, then, that the action of the acidic polymers is to substitute for an effect mediated by polymorphonuclear leukocytes. Perhaps, in the 24-hour period following the first of two doses of endotoxin, an acid polysaccharide (or other polymer) of large molecular size is released from these cells. At the end of this time, the function served by the second injection of endotoxin is to produce, transiently, the formation of unstable precipitable fibrinogen at the time when optimal levels of acid polymer exist for its precipitation in the circulating blood. Thus, the reason why two doses of endotoxin, spaced 24 hours apart, are necessary to produce the generalized Shwartzman reaction may be that the two main events which determine fibrinoid formation do not occur in phase with each other after one injection, even though both do occur. Precipitable fibrinogen appears within an hour or less after each injection but only lasts for about four hours; the postulated acidic polymer is not released from polymorphonuclear leukocytes in adequate amounts until 24 hours; therefore, a second dose of endotoxin is needed to bring about the reappearance of precipitable fibrinogen at the proper time for reaction with the polymer With use of Liquoid or dextran sulfate, one of the events can be created artificially at just the time when precipitable fibrinogen is abundantly present, and only one dose of endotoxin is therefore necessary.

Craig and Gitlin [41] have recently presented immunohistochemical evidence for the presence of fibrinogen or fibrin as a major component of fibrinoid in several human "collagen" diseases. Whether precipitation by tissue-derived acid polysaccharide is involved in the formation of these deposits is not known, but since the presence of such polysaccharides in fibrinoid is well recognized, the possibility merits further investigation.

The Role of Epinephrine in the Actions of Endotoxin

In a series of studies beginning in 1941, Delaunay and Boquet and their associates [42,43] described the reactions of peripheral arterioles which are characteristic of the early stages of the systemic response to endotoxin. Within 15 minutes after intravenous injection in rabbits, the authors observed intense prolonged arteriolar constriction, interrupted periodically by brief waves of vasodilation. In animals given large shock-producing doses, the phase of arteriolar constriction was later replaced by one of extreme persistent arteriolar dilation, accompanied by slowing and stasis of capillary blood flow. The initial reaction of constriction was prevented by pretreatment with Dibenamine (N,N-dibenzyl-β-chloroethylamine). On the basis of these observations, it was suggested that the systemic effects of endotoxin were associated with a generalized sympathomimetic response analogous to stimulation by epinephrine.

There are other reasons for suspecting that epinephrine, or a substance resembling epinephrine, may be implicated in the response to endotoxin.[2] Hyperglycemia, sometimes followed by hypoglycemia, is regularly produced by both endotoxin and epinephrine and, according to Boquet and Izard,[44] can be prevented by dihydroergotamine. Similarly, depletion of liver glycogen and elevated blood levels of lactic and pyruvic acid occur in response to both agents.

Additional points of resemblance include the production of leukocytosis (although the leukopenia which occurs during the first hour after endotoxin is not produced by epinephrine); interference with diapedesis of leukocytes into inflamed tissues [42]; the occurrence of petechial hemorrhages and focal necrosis in the walls of the stomach and upper gastrointestinal tract, mesentric lymph nodes, spleen, and liver,[43,45] and fibrinoid necrosis in the walls of the coronary arteries.[29,46]

It might be postulated, then, that the systemic effect of endotoxin is either to in-

crease the reactivity of terminal vessels to epinephrine or to increase the amount of epinephrine released by the adrenal medulla or liberated in peripheral tissues. The first possibility is supported by the findings described below, which suggest that alteration of vascular reactivity to epinephrine may be the basis of some of the pathophysiologic effects of endotoxin.[4,47]

Necrotizing Action of Epinephrine in Presence of Endotoxin.—When epinephrine is injected intradermally into normal rabbits, a transient reaction of local pallor occurs regularly, sometimes followed after a few hours by secondary hyperemia. No evidence of tissue destruction is demonstrable, however, even when large doses (100μg. to 200μg.) are administered repeatedly in the same skin site.

In contrast, epinephrine produces extraordinary lesions of hemorrhagic necrosis in the skin of rabbits which have received an intravenous injection of endotoxin at any time during the preceding four hours.[4] For example, when an animal is given an intravenous injection of 1μg. of purified endotoxin and an hour later 10μg. of epinephrine is injected into the abdominal skin in a volume of 0.2 cc., an oval or circular area of purple-black hemorrhage and necrosis measuring 6 to 8 cm. in diameter subsequently develops at the injection skin site. These lesions begin as a meshwork of engorged superficial vessels, resembling telangiectases, which appear three or four hours after the injection of epinephrine. During the next few hours, small petechiae begin to occur, and after about 12 hours the entire area becomes occupied by confluent hemorrhages.

These lesions seem to be due to a direct action of endotoxin on the blood vessels of the skin, altering their subsequent reactivity to the epinephrine. They do not represent secondary manifestations of fever, shock, or other systemic responses to endotoxin. This is shown by the fact that the same type of lesion is produced by an intradermal injection of a mixture of endotoxin and epinephrine, in which case endotoxin is not absorbed and none of its systemic actions come into play.[4]

Similar lesions are produced by arterenol in combination with endotoxin, but not by ephedrine, vasopressin (Pitressin), or serotonin.

Although the epinephrine lesions resemble local Shwartzman reactions in their gross aspects, they appear to involve quite different mechanisms. Heparin and mechlorethamine hydrochloride, which prevent the Shwartzman reaction,[26,27,40] do not prevent the epinephrine lesions, while phenoxybenzamine, chlorpromazine, and cortisone, which have no effect on the Shwartzman reaction, provide complete protection against the epinephrine lesion.[4] Histologically, the two differ sharply; in the Shwartzman reaction, there is a vigorous local inflammatory reaction, and the capillaries and venules are plugged by masses of leukocytes and platelets,[48] while the epinephrine lesions show widespread necrosis and hemorrhage without evidence of inflammatory-cell involvement.

Changes in Vascular Reactivity to Epinephrine Caused by Endotoxin.—In order to learn more about the mechanisms involved in the necrotizing effect of epinephrine described above, two techniques for quantitative measurement of vascular reactivity to epinephrine were utilized. One was the method originated by Katz [49] for measuring resistance to perfused fluid in the isolated rabbit ear, the other, the procedure of Zweifach [50] for visualizing the reactions of terminal vessels in the exteriorized mesoappendix of rats. With both techniques, it was found that small doses of endotoxin cause marked enhancement of vasoconstrictor reactions to epinephrine, while large doses bring about reversal of this effect and complete failure of all normal constrictor activity.[47]

In the experiments with the isolated perfused rabbit ear, the perfusion of relatively small amounts of endotoxin (0.5μg.) was followed within a few minutes by a two-hundred- to eight-hundred-fifty-fold poten-

tiation of the normal epinephrine response, as estimated by reduction of the effective threshold dose of epinephrine. But with a large dose of endotoxin (25μg. to 40μg.), the opposite effect occurred; instead of enhanced vasoconstriction, the test dose of epinephrine now produced immediate and extreme vasodilation. Similar biphasic changes in reactivity were observed with arterenol after endotoxin, but not with vasopressin or serotonin.

Analogous results were obtained in the in vivo experiments with the rat mesoappendix. Since rats are much more resistant than rabbits to endotoxin, larger amounts were employed. When sublethal doses (25μg. to 50μg.) were injected by vein, the constrictor reaction to epinephrine was potentiated as much as one-thousand-fold. The effect appeared within less than 30 minutes after injection of endotoxin and lasted about one hour. With a large dose of endotoxin, sufficient to produce shock (1 to 2 mg.), the enhancement of vasoconstriction was brief in duration or failed to occur at all, and instead the arterioles became, within an hour or less, completely unresponsive to massive doses of topically applied epinephrine. At this time, a phenomenon was observed which seems to shed light on the mechanism underlying the hemorrhagic skin lesions described in the preceding section. As the arterioles lost their capacity to respond to epinephrine, they gradually became widely dilated, but the venules remained tightly constricted after each application of epinephrine, with the result that large amounts of sluggishly moving blood were entrapped in the engorged capillary bed. At this time, each application of epinephrine was followed by showers of petechiae, which appeared within a few minutes throughout the site of application. Similar results were obtained with arterenol.

These events—loss of arteriolar reactivity with retention of venular constriction, pooling of blood in engorged capillaries, anoxia, and, finally, rupture of the capillary walls and hemorrhage—could reasonably account for the sequence of events observed in rabbit skin after the combined injection of endotoxin and epinephrine. In the latter case, it will be recalled that the earliest change to be seen in the skin was a dilated meshwork of small vessels, followed by the development of petechiae and confluent hemorrhage.

The similarities between the systemic reactions produced by endotoxin and epinephrine noted by Delaunay and Boquet [42,43] might also be accounted for on the basis of these observations. It is known that both epinephrine and arterenol are produced and stored in tissues, independent of the normal output of the adrenal medulla,[51] and it is safe to assume that the normal tone of terminal blood vessels, as well as the amount and rate of blood flow throughout the capillaries are governed to an important extent by the presence of catechol amines in the tissues. The biphasic changes in vascular reactivity produced by endotoxin could result in different types and degrees of tissue damage in different tissues, depending on the local concentration of pressor amines and the dose of endotoxin employed, with ischemia due to prolonged vasoconstriction in one area or with vasomotor collapse and hemorrhagic necrosis in another.

It must be recognized that although the reactivity changes are the same with arterenol as with epinephrine in the skin, ear, and mesoappendiceal models used in these experiments, the vascular responses to the two hormones in other organs are known to be quite different. Whether one or the other is of more importance in determining the site of character of tissue damage with endotoxin is a problem beyond speculation at the present time.

The physiologic basis for the observed changes in epinephrine reactivity remains unsettled. A possible, although insubstantial, clue is provided by recent observations which seem to implicate serotonin in the reaction. It has been learned that Compound 48/80 (condensation product of *p*-methoxyphenylethyl-methylamine and formaldehyde),

which is known to cause depletion of serotonin as well as histamine from mast cells and platelets will protect rats against lethal doses of endotoxin. Furthermore, topical application of epinephrine on the mesoappendix of rats given serotonin by vein, as well as application of a mixture of serotonin and epinephrine, results in vasomotor reactions which are strikingly similar to those seen with epinephrine in endotoxin-treated rats. Finally, the intradermal injection of a mixture of epinephrine and serotonin in rabbits has been observed to cause local reactions of hemorrhagic necrosis which resemble the skin lesions produced by epinephrine in endotoxin-treated rabbits. Thus, the possibility exists that at least part of the reaction to endotoxin may involve the release, in the immediate vicinity of blood vessel walls, of serotonin.

Summary and Conclusions

The observations reviewed above suggest the following generalizations concerning the physiopathologic alterations caused by the endotoxins of Gram-negative bacteria.

The reticuloendothelial system appears to function as a protective mechanism against endotoxin, although the way in which protection is mediated is by no means clear. Furthermore, in the course of systemic intoxication by endotoxin, the reticuloendothelial cells are themselves damaged. Tolerance to endotoxin may represent an enhanced activity on the part of these cells.

The primary shock-producing lethal action of endotoxin involves mechanisms which differ from those involved in the local and generalized Shwartzman reactions. The two types of response can be experimentally dissociated by treatment with cortisone, chlorpromazine, or phenoxybenzamine. Dissociation is also demonstrable in rabbits of different ages; young rabbits are resistant to the lethal effect but susceptible to the Shwartzman reaction, while the reverse is true in mature animals.

The intravascular fibrinoid which occludes the glomerular capillaries in the generalized Shwartzman reaction and leads to the development of bilateral cortical necrosis of the kidneys appears to be derived from the fibrinogen of the circulating blood. The two stages of the reaction are believed to be partial polymerization of fibrinogen (manifested by its heparin-precipitability following an injection of endotoxin) and the liberation of an acid polymer of large molecular size from leukocytes (which brings about intravascular precipitation of the altered fibrinogen). Coexistence of these two participants in the blood at the same time requires that two appropriately spaced injections of endotoxin be given. The events can be imitated by the administration of a synthetic heparin-like polymer within one hour after endotoxin.

Some of the local and systemic reactions to endotoxin may be due to alterations of the reactivity of terminal blood vessels to epinephrine. In the presence of endotoxin, epinephrine is capable of producing extensive lesions of hemorrhagic necrosis in the rabbit. It is possible that the biphasic changes in epinephrine reactivity demonstrable in terminal vessels may play a determining role in the pathogenesis of endotoxin shock.

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Cold Agglutinins

VIII. Occurrence of Cold Isohemagglutinins in Patients with Primary Atypical Pneumonia or Influenza Viral Infection, Boston City Hospital, June, 1950, to July, 1956

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The availability of new methods for the isolation and study of viral diseases and the discovery of new groups of viruses related to nonbacterial respiratory infections with the use of these methods [1] have shed some light on the etiology of a small proportion of the infections that have been recognized under the vague name "primary atypical pneumonia," commonly referred to as "virus pneumonia." In 1943, Peterson, Ham, and Finland,[2] after encountering three patients with acute hemolytic anemia associated with cold agglutinins (autohemagglutinins) among such cases, studied this phenomenon and found that the great majority of cases of primary atypical pneumonia seen at the Boston City Hospital during that season showed significant titers of cold isohemagglutinins in their serum. At about the same time, Turner [3] made a similar observation among military personnel stationed in England.

More extensive studies by Turner and Finland and their respective co-workers [4-6] indicated that among cases of various acute respiratory infections significant titers of cold agglutinins developed only in patients with primary atypical pneumonia. Isolated instances of cold hemagglutination in cases of pneumonia which, retrospectively, could be categorized as primary atypical pneumonia had been described previously,[7-9] but the reports referred to were the first to relate the phenomenon of cold hemagglutination to the disease and to suggest that it was in some way related to its still unknown etiology, which was presumed to be some virus or viruses.

Since the publication of these early reports, the phenomenon of cold hemagglutination in primary atypical pneumonia has been confirmed by many observers,[10-17] and although most of them have attributed some diagnostic value to the test for cold agglutinins others have not. Cold hemagglutinins in general, but particularly in relation to hemolytic anemias, including those that occur in atypical pneumonia, have also been the subject of an extensive review by Stats and Wasserman.[18] These have also been reviewed more recently by others.[19,20]

There have also been a number of studies on the etiologic relationship of the new groups of viruses, particularly the adenoviruses, to acute respiratory diseases.[21-27] In the course of these studies, although cases of atypical pneumonia have been encountered from which adenoviruses (Types 3, 4, and 7) have been isolated, or their relation to the disease confirmed serologically, none of these cases was associated with the development of cold agglutinins. This indicated, as was suggested earlier, that cases of primary atypical pneumonia in which cold hemagglutinins develop in the serum may be associated with one or more specific viruses that are different from the ones that cause most of the cases not associated with this phenomenon; the discrepancies in the findings of different observers are

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compatible with such an explanation. A continued search for other viruses that may be etiologically related to the types of cases in which cold agglutinins develop is therefore indicated, provided, of course, that such cases are still prevalent.

In the first detailed report from Boston [6] it was noted that such cases were encountered in large numbers during the autumn and winter of 1942-1943 and were much less frequent during the following year; in both years, the incidence was lowest during the spring. In a later report [28] it was shown that cases of atypical pneumonia associated with the development of cold agglutinins were encountered in each of four successive years ending June, 1950; the frequency with which cold agglutinins were encountered in cases considered to be nonbacterial atypical pneumonias in these years varied from 16% to 33% and again were most frequent in the fall and least frequent in the spring. In the present paper, it is indicated that such cases have been occurring in Boston during the six years after 1950 (through June, 1956), and so the continued search for a viral agent in these cases is still justified.

Patients and Methods

All but a few of the patients studied were admitted for treatment on the Medical Services of the Boston City Hospital; the rest were from the Pediatric Wards of the same hospital or from members of the hospital staff. The types of cases included in this study and the method of testing for cold agglutinins were the same as in the previous report.[28]

Results

The frequency with which cold isohemagglutinins were demonstrated in significant titers (1:40 or higher) among patients with primary atypical pneumonia observed at the Boston City Hospital between July, 1950, and June, 1956, is shown in Table 1. The diagnosis was made in these cases on clinical grounds and was confirmed by roentgenograms. No significant bacterial etiology was demonstrated in these cases. In each of these cases at least two specimens of serum were tested simultaneously, and in nearly every instance a fourfold or greater rise in titer of cold hemagglutinins was demonstrated during or after the middle of the second week of the disease or a fall in titer from a high level was observed during convalescence. In each instance at least 2+ agglutination (coarse clumping grossly visible to the naked eye and not dispersed by gentle shaking) was present in 1:20 dilution of serum. A few patients in whom persistently elevated titers were made out early in convalescence are also included.

TABLE 1.—*Occurrence of Significant Changes in Titers of Cold Isohemagglutinins in Patients with Primary Atypical Pneumonia*

		July-September	October-December	January-March	April-June	Total
1950–1951	No. tested	14	23	10	7	54
	No. positive *	2	3	2	1	8
	% positive	*14*	*13*	*20*	*14*	*15*
1951–1952	No. tested	11	22	8	2	43
	No. positive	3	3	0	0	6
	% positive	*27*	*14*	*0*	*0*	*14*
1952–1953	No. tested	3	6	8	5	22
	No. positive	1	1	1	0	3
	% positive	*33*	*17*	*13*	*0*	*14*
1953–1954	No. tested	7	8	9	5	29
	No. positive	3	4	4	0	11
	% positive	*43*	*50*	*44*	*0*	*38*
1954–1955	No. tested	7	35	36	10	88
	No. positive	3	13	9	1	26
	% positive	*43*	*37*	*25*	*10*	*30*
1955–1956	No. tested	7	7	10	0	24
	No. positive	2	1	1	0	4
	% positive	*29*	*14*	*10*	..	*17*
1950–1956	No. tested	49	101	81	29	260
	No. positive	14	25	17	2	58
	% positive	*29*	*25*	*21*	*7*	*22*

* Fourfold or greater change (rise early or fall late in convalescence).

It is seen that patients with atypical pneumonia associated with the development of cold isohemagglutinins were observed in each of the six years. During every one of these years, such cases occurred during the summer and autumn (July through December), and in all but one of these years at least one case was observed during the winter months (January through March); however, only an occasional case was noted during the months of April through June. Although the total number of cases is too few and the manner in which they were selected is not suitable for significant com-

TABLE 2.—*Occurrence of Significant Titers* of Cold Isohemagglutinins in Two Groups of Patients*

Year	Patients with Primary Atypical Pneumonia; Single Specimen Obtained During Convalescence			Patients with Serologically Proved Influenza and Significant Change in Titer of Cold Agglutinins		
	No. Tested	No. Positive	% Positive	No. Tested	No. Positive	% Positive
1950–1951	31	1	3	72	1	1.3
1951–1952	12	0	0	26	0	0
1952–1953	9	1	11	7	0	0
1953–1954	16	2	13	0	0	
1954–1955	31	4	13	39	4	10.2
1955–1956	13	1	8	0	0	
Total	112	9	8	144	5	3.4

* Titer of 1:40 or higher.

parisons, the proportion of cases with cold agglutinins did not seem to vary directly with the numbers of cases in which the diagnosis of atypical pneumonia was made in the different years. Throughout the entire period of six years, about one in five patients so diagnosed yielded cold agglutinins in the serum, and the proportion varied in the different years from one in seven to slightly more than one in three.

In Table 2 are shown the results of tests for cold agglutinins in the serum of two additional groups of patients. In one group, of 112 patients, a single specimen of serum was obtained during convalescence, usually more than three weeks after the onset of symptoms considered retrospectively to be consistent with a diagnosis of primary atypical pneumonia. Some of these patients were admitted for other illnesses or for bacterial complications of the respiratory tract. Only nine of these patients had significantly elevated titers of cold isohemagglutinins.

The second group consisted of 144 patients in whom a diagnosis of influenza viral infection was established by the demonstration of a fourfold or greater rise in the inhibiting titer of their serum for chicken-cell agglutinins by either the influenza A or influenza B viruses that were prevalent at the time; an influenza virus of the corresponding type was also isolated from throat washings of many of these patients. Four of the five cases of proved influenza viral infection and significantly elevated titers or changes in titer of cold isohemagglutinins occurred in 1954-1955, when there were also the largest numbers of patients with primary atypical pneumonia and also the largest number with cold agglutinins. This suggested the possibility that these patients may have had both infections. In three of these patients the titers of cold agglutinins dropped from high levels, and in a fourth the high titers persisted while the titers of specific influenza viral antibodies were rising; the history in these cases suggested the likelihood of an early illness consistent with a diagnosis of primary atypical pneumonia and a more recent attack of clinical influenza.

The distribution of titers of cold isohemagglutinins in all of the positive cases is shown in Table 3. This distribution was similar among patients with atypical pneumonia in whom only a single blood specimen was obtained during convalescence and in those in whom there were multiple specimens with changing titers. The distribution of titers in both of these groups is also similar to that observed with the same method in the cases of atypical pneumonia previously studied in this laboratory.[6] The single patient listed as having a titer of 1:20 had strong (2+) agglutination in that dilution of the serum that was obtained late in the febrile course, and no agglutination was demonstrated in lower dilutions in earlier specimens; the patient left the hospital, and further specimens could not be

TABLE 3.—*Distribution of Maximum Titers of Cold Isohemagglutinins in Three Groups of Patients*

Maximum Titer (Reciprocal)	Group A *		Group B *	Group C *
	Patients, No.	%	Patients, No.	Patients, No.
20	1	1.7	—†	—†
40	7	12.1	2	3
80	8	13.8	2	1
160	12	20.7	2	1
320	14	24.1	2	0
640	9	15.5	0	0
1280	7	12.1	1	0
Total	58	100	9	5

* A, primary atypical pneumonia with significant changes in titer of cold agglutinins;
B, primary atypical pneumonia with high titer in single specimen during convalescence;
C, serologically proved influenza and significant change in titer of cold agglutinins.
† Excluded from this group.

TABLE 4.—*Nonspecific Changes in Titer of Antihemagglutinins for Influenza Viruses Associated with Elevated or Changing Titers of Cold Isohemagglutinins in Serums of Patients with Primary Atypical Pneumonia*

Case	Day of Illness	Titer * of Cold Isohemagglutinin	Inhibiting Titer * of Chicken-Cell Agglutination			
			Influenza A		Influenza B	
			FM1	53-13	BON	52-8
1	3	<10		16		16
	19	640		<4		<4
2	5	<10	4	32	16	4
	22	640	32	256	64	32
3	10	160	64		64	32
	16	160	256		256	256
4	7	10	512			64
	15	320	64			<4
5	14	320	128			32
	20	80	32			8
6	12	160	32		16	
	25	80	<4		<4	
7	10	80		4	<4	32
	16	80		16	16	128
8	5	<10	64	16		64
	14	80	<4	<4		<4

* Reciprocal or serum dilution.

obtained. Titers higher than 1:160 were not obtained in any of the serums from patients with proved influenza viral infection.

In the serums of almost all of the patients with primary atypical pneumonia, tests were also done for chicken-cell antihemagglutinins with standard strains of influenza A and B viruses and with strains recently isolated in this laboratory, in addition to the tests for cold isohemagglutinins. In nearly all of the cases of primary atypical pneumonia, other than the five possible cases already referred to that had specific influenza viral antibodies and cold agglutinins, no changes in titer of influenza viral antibodies occurred, regardless of whether or not cold isohemagglutinins developed. In a few patients, however, nonspecific changes in antihemagglutinin titers for influenza viruses were associated with elevated or changing titers of cold hemagglutinins. Some of the findings in these cases are listed in Table 4. Similar changes were noted for other strains of influenza viruses of these types, but this was not true for all of the strains of each of the types in every instance.

Summary and Comment

The data presented indicate that cases of primary atypical pneumonia associated with the development of high titers of cold isohemagglutinins have been occurring in Boston in each of the six years from July, 1950, through June, 1956, just as they had been occurring from 1942 to 1950. These cases have been occurring throughout most of the year, except that they were rarely observed during the months of April through June. The numbers of cases of primary atypical pneumonia with and without demonstrable cold agglutinins probably varied independently from year to year. The findings of other workers, which indicate that primary atypical pneumonia may be due to adenoviruses of certain types but that no such viruses have been shown to be etiologically related to cases in which cold agglutinins develop, are compatible with the suggestion that the latter cases may be due to other specific viral agents. Further search for new agents in such cases therefore seems indicated.

Data are also presented to show that in the serums from some patients with primary atypical pneumonia elevated or changing titers of cold isohemagglutinins may be associated with changes in titer of antihemagglutinins of influenza viruses which are not type-specific and apparently are not related to influenza viral infections. Some data in other patients, however, are compatible with concomitant or sequential infections with the agents of primary atypical pneumonia and the viruses of influenza.

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Antibiotic Therapy in the Management of Hepatic Coma

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Hepatic coma is a metabolic disturbance occurring in some patients with liver disease which manifests itself as a "disorder of consciousness." The clinical features of the syndrome have been defined by Adams and Foley,[1] providing criteria for diagnosis and a background for the investigation of its pathogenesis. The relationship between this disorder and toxic nitrogenous substances is generally recognized.[2] These toxic substances, including ammonia, are believed to be a product of bacterial enzymatic action within the intestine.[3] Antibacterial agents effective against intestinal organisms have been reported to improve prognosis in hepatic coma.[4,5,7] Assessment of results is complicated by the simultaneous utilization of a number of therapeutic measures and by the varied prognosis of the syndrome. Studies such as that reported by Fisher and Faloon[6] of patients with episodic stupor, whether spontaneous or following portacaval anastomosis, allow more controlled observations related to isolated therapeutic efforts. We wish to report observations relating to factors precipitating the syndrome in two patients with episodic stupor and the response to the administration of various antibiotics. In addition, observations were made

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TABLE 1.—*Hepatic Coma Associated with Hepatic Failure* *

Patient	Age, Yr.	Sex	Diagnosis	Duration of Coma †	Stage of Coma ‡	Serum Bilirubin, Mg./100 Ml.	Specific Therapy	Response to Therapy	Ultimate Result
3	54	M	Cirrhosis of alcoholic (autopsy)	3+ days	Impending	16.8	1. Arginine 2. Paromomycin 3. Enemata and purges 4. Protein restricted for 4 days	Improved	Died
4	64	F	Viral hepatitis (biopsy and autopsy)	3 days	Impending	18.0	1. Complete protein restriction 2. Enemata and purges 3. Neomycin 4. Hydrocortisone i. v. for last 6 days	None	Died
5	33	M	Cirrhosis of alcoholic	24 hr.	Impending	19.9	1. Enemata 2. Paromomycin 3. Protein restriction for 6 days	Improved	Recovered
6	38	M	Cirrhosis of alcoholic (autopsy)	3+ days	Impending	38.0	1. Enemata and purges 2. Paromomycin 3. Protein restricted for 14 days	None	Died
7	69	F	Cirrhosis of alcoholic (autopsy)	3+ days	Coma	24.2	1. Neomycin 2. Enemata and purges 3. Protein restriction for 10 days	Slight improvement	Died
8	44	M	Cirrhosis of alcoholic (autopsy)	3 days	Impending	20.0	1. Enemata and purges 2. Protein restricted 3. Paromomycin	Improved in spite of subsequent gastrointestinal bleeding	Died

* Ascites was present in all.
† Duration of coma prior to initiation of specific therapy.
‡ Stage of coma when treatment was initiated.

TABLE 2.—*Hepatic Coma Associated with Massive Gastrointestinal Hemorrhage* *

Patient	Age, Yr.	Sex	Precipitating Factor	Duration of Coma †	Stage of Coma ‡	Serum Bilirubin, Mg./100 Ml.	Ascites	Specific Therapy	Response to Therapy	Ultimate Result
9	39	M	Massive gastrointestinal hemorrhage uncontrolled for 5 days	24 hr.	Impending	3.2	+	1. Protein restricted for 11 days 2. Arginine 3. Paromomycin 4. Tamponage	Improved	Recovered
10	45	F	Massive gastrointestinal hemorrhage (controlled) (autopsy)	3 days	Coma	16.4	—	1. Protein restricted 2. Arginine 3. Transfusions 4. Enemata and purges 5. Paromomycin	Improved with first episode of coma	Died following recurrent hemorrhage, coma, and exploratory laparotomy
11	56	M	Massive gastrointestinal hemorrhage (controlled)	24 hr.	Impending	3.5	+	1. Tamponage 2. Enemata and purges 3. Protein restricted for 4 days 4. Paromomycin	Improved	Recovered

* All alcoholics with cirrhosis.
† Duration of coma prior to initiation of specific therapy.
‡ Stage of coma when treatment was initiated.

in a number of cases of acute hepatic coma associated with hepatic failure and/or massive gastrointestinal hemorrhage.

Materials and Methods

Patients Studied.—Eleven patients with liver disease and hepatic coma were studied on the medical and surgical wards of the Boston City Hospital and the Thorndike metabolic ward. Data relating to clinical status and laboratory measurements are included in Tables 1 and 2. Nine patients had cirrhosis associated with chronic alcoholism; one had cirrhosis of unknown etiology, and one had viral hepatitis. Clinical and biochemical findings were supplemented by histologic evidence from liver biopsy or at autopsy in the case of hepatitis and in 8 of the 10 cases with cirrhosis.

Clinical Evaluation of Coma.—The criteria for diagnosis of hepatic coma were those of Adams and Foley.[1] The following grades were recognized: Grade 1, minimal signs including a "flapping" tremor of the hyperextended hands, lethargy, slurred speech, inability to perform simple arithmetic problems, and failure to maintain a neat habitus where this was normally observed, but maintaining orientation. Grade 2, the above findings accentuated, in addition to which the patients exhibited disorientation and confusion but maintained sphincter control. Grade 3, a state of stupor from which the patient could be aroused to produce motion or incoherent speech, along with

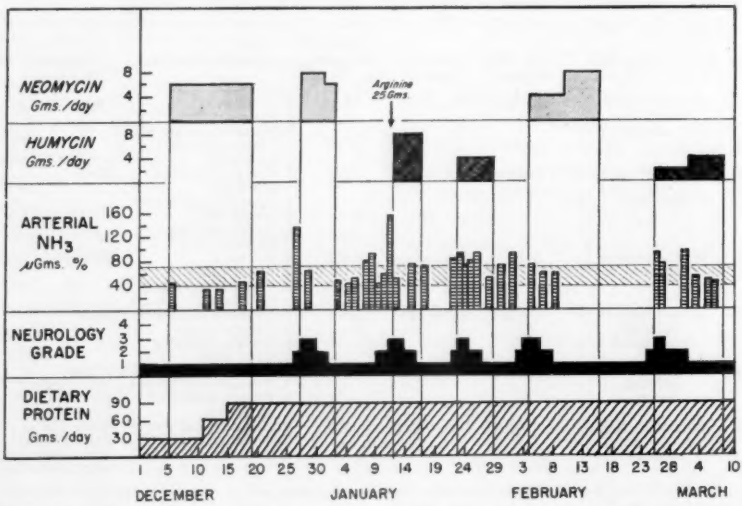

Fig. 1 (Case 1).—Portal cirrhosis in a 54-year-old man. Response to alterations in dietary protein and administration of antibiotics in a case of spontaneous episodic stupor.

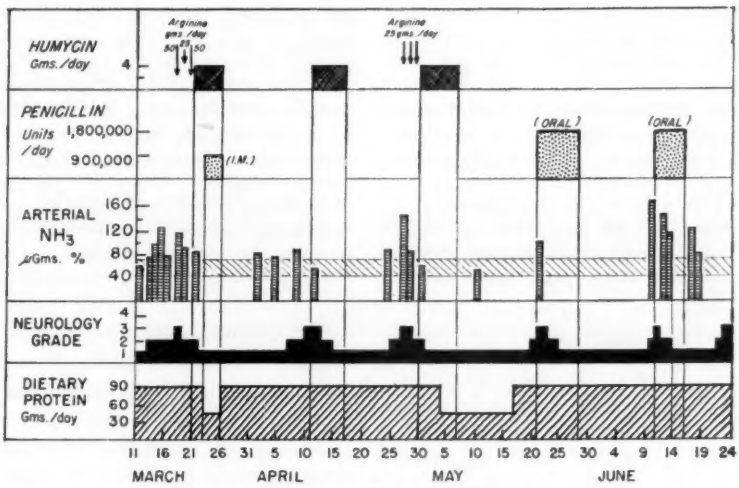

Fig. 2 (Case 1).—Same as in Figure 1.

incontinence. Grade 4, deep coma. Significant improvement has been defined as recovery of consciousness in an originally stuporous or comatose patient and complete recovery in a patient with impending hepatic coma.

Groups of Patients.—The patients were divided into three groups. Group 1 consisted of two patients with episodic stupor who were studied under the controlled conditions of a metabolic ward. A case report of one follows. The other patient (Case 2) a 57-year-old woman, had cirrhosis of unknown etiology and a portacaval anastomosis. The development of coma and the responses to treatment are indicated in Figures 1 to 3. Group 2 consisted of six patients mainfesting progressive hepatic failure, as indicated by elevated serum bilirubin concentrations, the presence of ascites, and the spontaneous appearance of hepatic coma. Group 3 consisted of three patients with hepatic coma for whom massive gastrointestinal hemorrhage was the precipitating factor.

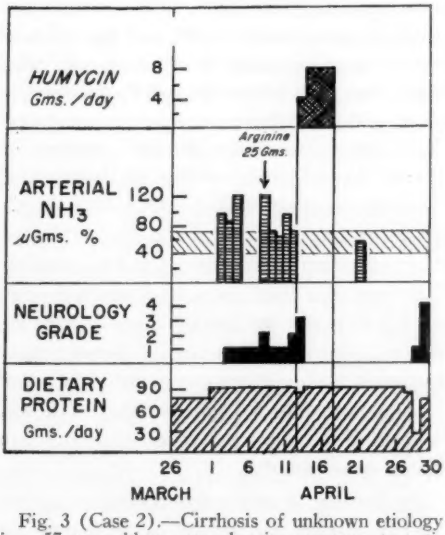

Fig. 3 (Case 2).—Cirrhosis of unknown etiology in a 57-year-old woman, showing response to antibiotics in a patient with portacaval anastomosis.

Treatment.—Studies in Group 1 included observations related to the effects of administration of varied amounts of dietary protein and of several antibiotics in varying dosage. These will be discussed in detail later. Patients in Groups 2 and 3 were treated according to a standard therapeutic regimen, as previously described.[7] This includes purges, enemata, the exclusion of sedatives and other drugs known to cause or aggravate hepatic coma, and the elimination of dietary protein. Initially, dietary protein was excluded entirely until definite evidence of improvement existed. More recently, dietary protein in restricted amounts, usually in the form of a sodium-free powdered milk preparation (Lonalac*) (300 gm.) or a quart of low-sodium milk, was instituted 48 to 72 hours after the commencement of antibiotic therapy. A daily intake of about 1500 Cal. was maintained by oral or, if necessary, intragastric administration of a partially hydrolyzed starch, Dexin.† Control of variceal bleeding was by

* Lonalac was provided by Mead Johnson & Company, Evansville, Ind.

† Burroughs Wellcome Company, Inc., Tuckahoe, N. Y.

balloon tamponage. Neomycin (Mycifradin‡) sulfate or Paromomycin (Humycin§) sulfate was administered in daily doses of 1 gm. or more, four times daily.

Biochemical Methods.—Tests of liver function and serum electrolyte determinations were performed in every patient, with use of standard methods reported earlier.[8] Blood ammonia concentrations were measured by a modification[9] of Conway's technique.[10] All determinations reported here were done on arterial blood samples. Normal values in this laboratory range from 40μg. to 70μg. per 100 cc.

Results

Group 1.—This group consisted of patients manifesting episodic stupor. Extensive observations were made on a patient with cirrhosis of alcoholics who manifested marked protein intolerance presumably associated with extensive portal-systemic collateral circulation. A brief case report follows.

CASE 1.—A 54-year-old white male laborer was admitted to the hospital for treatment of an accidentally sustained burn on the arm on Oct. 4, 1956. Systemic inquiry elicited some recent weight loss as the only other symptom. A history of prolonged excessive intake of alcohol, along with a poor dietary intake, was obtained. Examination revealed a moderate degree of jaundice, multiple spider nevi, hepatomegaly, splenomegaly, and evidence of peripheral neuritis. In addition, he was noted to be lethargic, drowsy, and disoriented with respect to time, and he had difficulty with simple arithmetic problems. A flapping tremor was demonstrated. Initial laboratory investigations revealed serum bilirubin of 8.6 mg. per 100 cc.; cephalin flocculation, 4+; formol gel test, 4+; stool guaic test, negative; hematocrit, 35%. A clinical diagnosis of cirrhosis of the alcoholic with impending hepatic coma was made. Following protein withdrawal, purging and enemata, and the administration of tetracycline, the patient showed rapid improvement of mental status. On institution of a normal house diet (protein content, 60-70 gm.), the patient soon developed a recurrence of impending hepatic coma. This intolerance to dietary protein persisted for more than eight months in spite of gradual improvement in liver function during this time as indicated by decreased serum bilirubin and flocculation values. Toward the end of the study an increased tolerance to dietary protein was noted. Persistent hypoprothrombinemia contraindicated liver biopsy or splenoportography. Thirty-six days following admission the patient was transferred to the Thorndike metabolic ward, where he was observed continuously over a period of seven months. During this time he had 11 episodes of severe impending coma.

The following observations were made in this patient and Patient 2.

Effect of Dietary Protein: The development of impending hepatic coma in Patient 1 was clearly related to the quantity of dietary protein as shown in Figure 1. On an intake of 30.0 gm. minimal neurologic and mental signs could be elicited. Subsequently, on a constant daily intake of 90.0 gm. of protein, the absence of oral antibiotics repeatedly produced severe impending coma.

Effect of Antibiotics: Neomycin was administered to Patient 1 on three occasions. Initially 6.0 gm. daily was given while dietary protein was gradually increased from 30.0 to 90.0 gm., and no alteration in mental status was noted. Later neomycin and Paromomycin were given when severe impending coma was evident, but the protein intake was maintained constant. It was found that both neomycin and Paromomycin when given in doses of 4.0 gm. or more daily for 5- to 6-day periods resulted in clinical improvement within 48 hours and complete recovery within 4 to 5 days. Signs of impending coma recurred 7 to 10 days after cessation of therapy. On one occasion Paromomycin was given in a daily dosage of 2.0 gm. for six days. Some degree of clinical improvement occurred, but complete recovery was not apparent until the dose was increased to 4.0 gm. per day. No significant differences in response to the two drugs were noted.

In Patient 2 severe impending coma occurred during an intake of 90.0 gm. of protein. A dramatic response and complete

‡ Mycifradin provided by The Upjohn Company, Kalamazoo, Mich.; 1.0 gm. is the equivalent of 0.70 gm. of neomycin base.

§ Humycin (Paromomycin Sulfate) was provided by Parke, Davis & Company, Detroit; 1.0 gm. is the equivalent of 0.60 gm. of paromomycin base. Recently Paromomycin Sulfate has been labeled Humatin Sulfate; 4 capsules are the equivalent of 0.60 gm. Paromomycin Sulfate.

recovery occurred within 24 hours of the administration of 4.0 gm. of Paromomycin (Fig. 3). Further treatment (8.0 gm. daily for four days) was given. Clinical signs of impending coma occurred 11 days after cessation of therapy.

On one occasion the concomitant administration of penicillin (300,000 units intramuscularly twice daily for three days) with Paromomycin in Patient 1 for a complicating staphylococcal skin infection was followed by a remission lasting 12 days. Later a course of oral penicillin (Pentids, Squibb) in a dose of 400,000 units three times daily for seven days in the presence of severe impending coma resulted in clinical recovery within 4 days and recurrence of the syndrome 17 days after completion of treatment. Penicillin treatment in the same dosage over a five-day period was repeated during a subsequent episode of impending coma. Clinical recovery occurred within three days, and recurrence, within seven days.

Relationship to Arterial Ammonia Concentrations: Determinations of arterial ammonia concentrations were done frequently in Patient 1. As indicated in Figures 1 and 2, these values tended to parallel the neurological grade of the patient. Values greater than 70.0μg. per 100 cc. usually were found in the presence of impending coma, and normal values, with clinical remission. This was, however, not a consistent finding, and the degree of elevation did not correlate with the depth of coma.

Group 2.—This group consisted of six patients in whom hepatic coma was a manifestation of hepatic failure, as expressed by the criteria of moderate to severe jaundice and the presence of ascites. Their clinical status, treatment, and the results are shown in Table 1. Complete recovery occurred in only one patient. Coma developed spontaneously in Patient 8, and striking improvement occurred with treatment in spite of a subsequent moderately severe gastrointestinal hemorrhage. Death occurred three days later and was probably related to the injudicious administration of excess saline parenterally. Patient 3 showed definite evidence of improved mental status with treatment but later developed signs of renal insufficiency, oliguria, rising blood nonprotein nitrogen concentration, and uncontrollable though moderate gastrointestinal bleeding and died. In the other three patients hepatic coma progressed in spite of treatment and death ensued.

Group 3.—Group 3 consisted of three patients in whom coma followed massive gastrointestinal hemorrhage, presumably as a result of rupture of esophageal varices. Their clinical status, treatment, and results are shown in Table 2. Patient 10 was admitted comatose following massive gastrointestinal hemorrhage but also had moderately severe liver-cell damage as indicated by a serum bilirubin value of 16.4 mg. per 100 cc. Complete recovery followed control of bleeding, protein deprivation, and Paromomycin administration. Bleeding, however, recurred; coma ensued, and the patient died after emergency exploratory laparotomy. Patient 9 was admitted to the hospital in a stuporous state after a moderate hemorrhage. Further bleeding occurred, and the patient lapsed into coma. The complete therapeutic regimen as described earlier was instituted, but complete recovery did not occur until 48 hours after bleeding was finally controlled. Patient 11 was admitted stuporous after massive bleeding. The bleeding was controlled by tamponage; protein was not allowed, and enemata and purges were given. Some improvement of mental status was noted at the time when Paromomycin therapy was started, 12 hours later. The patient continued to show satisfactory recovery, and dietary protein intake was instituted several days later, without deleterious effect.

Comment

Intolerance to dietary protein in dogs with a portacaval shunt (Eck fistula) was demonstrated in the latter part of the 19th century.[11] Forced meat feeding resulted in a characteristic neurologic syndrome with

irritability, disequilibrium, convulsions, coma, and death. The administration of nitrogenous substances, including dietary protein, by Phillips et al.[12] to certain patients with cirrhosis resulted in a syndrome indistinguishable from spontaneous hepatic coma. They clearly showed that increasing the dietary protein produced the syndrome, while protein restriction resulted in recovery. The characteristic syndrome of episodic stupor was described by McDermott[9] in a patient with portacaval anastomosis. In 1954 Sherlock et al.[13] described five patients with chronic hepatic coma, "portal-systemic encephalopathy," associated with hepatic cirrhosis and an extensive portal-systemic collateral circulation in whom intolerance to dietary protein was again clearly shown. The recent study of Fisher and Faloon[6] and our own observations in two patients with episodic stupor confirm the relationship of dietary protein to the genesis of hepatic coma.

Attempts have been made to treat hepatic coma by administration of antibiotics. Farquhar[4] found that chlortetracycline benefited three of four patients with hepatic coma. Phear et al.[14] showed that chlortetracycline would prevent the development of neurological disturbances provoked by methionine in certain patients with cirrhosis. Fisher and Faloon[6] showed that oral neomycin in doses of 10.0 gm. or greater daily improved or prevented hepatic coma in a patient with portacaval anastomosis in spite of continued protein intake. Our own studies in two patients with episodic stupor again demonstrate that coma can be prevented or eliminated by the administration of antibiotics, including neomycin, Paromomycin, and penicillin in spite of continued intake of nitrogenous substances.

It seems reasonable to conclude that toxic metabolites derived from dietary nitrogenous substances which the liver fails to detoxify or which by-pass the liver through a portal-systemic collateral circulation are etiologic factors in the genesis of episodic stupor and in those cases of hepatic coma precipitated by massive gastrointestinal hemorrhage and are at least important contributing factors in all cases of hepatic coma. The striking response to nonabsorbable antibiotics further strongly suggests that the metabolites are produced by bacterial action on nitrogenous material in the intestines.

The exact nature of these toxins has not been established. The role of ammonia, however, has been extensively explored. Ammonia in high concentration occurs normally in portal blood[15] and is converted to urea in the liver.[16] In patients with poor liver function and/or portal-systemic collateral circulation, as in man or dog with portacaval anastomosis, ammonia may by-pass the liver.[17] That ammonia in the peripheral circulation may produce toxic effects even in the absence of liver disease has been shown in animals[18,19] and has been reported as a complication of enthusiastic treatment of metabolic alkalosis with ammonium chloride in a patient with pyloric obstruction by Wolfe et al.[20] In the two patients with episodic stupor we have studied, a tendency for arterial ammonia concentration to parallel the clinical state was noted, but, as stated by others, the relationship was an inconsistent one and the depth of coma did not correlate with the degree of elevation. Furthermore, treatment with glutamic acid,[21] aspartic acid,[7] or arginine,[20] as well as a regimen designed to eliminate nitrogenous material from the intestines,[7] may result in a fall of arterial ammonia levels but is associated with variable clinical effects. Toxic substances other than ammonia may therefore be important. Methionine may produce impending hepatic coma in sensitive persons with inconstant elevations of ammonia levels.[22] It has been suggested that a breakdown product of methionine, such as methyl hydrosulfide, or methylmercaptan, may be the toxic factor. Other toxic substances, such as indoles,[23] phenols,[24] and amines,[25] are also formed by bacterial action on nitrogenous substances in the intestine and may be of clinical significance.

The response to arginine as reported by Wolfe[20] and to antibiotics in the two patients with episodic stupor studied are of considerable interest. Patient 2 demonstrated a striking and dramatic response to the intravenous administration of arginine, whereas Patient 1 showed only minimal improvement in spite of continued arginine therapy and marked reduction in blood ammonia. Both patients, however, demonstrated prompt clinical recovery with the administration of antibiotics. These observations would suggest that perhaps several toxic factors in combination, of which one is ammonia, play a role in the genesis of the syndrome. Ammonia intoxication may be the predominant factor in some patients, such as Patient 2, who have shown prompt and striking response to ammonia-reducing substances. In other patients, such as Patient 1, ammonia may play a less prominent role and some other toxic factor, which does not respond to ammonia-removing substances (arginine), would be more important. As the toxic substances are probably derived from intestinal bacterial action, the administration of oral antibiotics would be expected to be of some therapeutic benefit.

The studies of Silen et al.[19] showed neomycin to be more effective than sulfasuxidine or tetracycline (Achromycin) in reducing the ammonia content of the portal venous system in dogs. Dintzis and Hastings[3] studied the urease activity of the intestinal tract of mice and demonstrated penicillin to be superior to either sulfaguanidine or oxytetracycline (Terramycin) in reducing this activity. Our controlled observations in Patient 1 have shown no superiority of one nonabsorbable antibiotic over another. The response to penicillin, not previously reported in man, though inferior to that with the other antibiotics, was somewhat unexpected. The studies of Phear et al.[22] showed that among the intestinal organisms with urease activity Aerobacter aerogenes and Proteus vulgaris, morganii, and mirabilis were most active in ammonium production. These organisms, with the exception of P. mirabilis, are relatively insensitive to penicillin.[26,27] However, the greater therapeutic effectiveness and the desirable characteristic of being poorly absorbed from the intestinal tract make neomycin and Paromomycin the antibiotics of choice.

The studies of Fisher and Faloon[6] indicated that doses in excess of 10.0 gm. of neomycin daily were required to treat or prevent hepatic coma in their patient with a portacaval anastomosis. We have found that a dose of 4.0 gm. daily was entirely adequate in reducing ammonia levels and producing clinical improvement and that no further benefit could be derived by increasing the dosage. As the clinical response persists for some weeks after therapy is stopped, patients with episodic stupor may be managed satisfactorily by allowing a regular diet with antibiotic therapy whenever it is necessary to control symptoms.

Assessment of the therapeutic value of any agent in hepatic coma is difficult, owing to the varied prognosis associated with the different factors which may contribute to the syndrome in any individual case. While in the controlled studies in the patients with episodic stupor therapeutic effectiveness of antibiotic therapy was clearly demonstrated, no such positive statement can be made regarding its use in the cases of coma associated with hepatic failure or massive gastrointestinal hemorrhage. These results clearly confirm previous observations that hepatic coma associated with hepatic failure carries a poor prognosis, whereas in other cases the prognosis is more closely related to the control of the precipitating factor.[7] However, it is reasonable to assume that the use of antibiotics to prevent the production of toxic metabolites would be of potential benefit in all cases. The therapeutic effectiveness of antibiotics is strongly suggested from the results of a controlled study in which a therapeutic regimen which included withdrawal of dietary protein and the administration of antibiotics was compared with a previous regimen where protein

was administered but antibiotics were not given.[7]

The administration of diets deficient in protein and other factors has resulted in the production of fatty liver, cirrhosis, and acute massive hepatic necrosis in rats.[28] Phillips et al.[29] have shown that the active cirrhosis of three chronic alcoholics failed to improve when these patients were provided with a purified diet (glucose and minerals), but subsequent improvement occurred when an adequate diet was provided for a similar period. These findings suggest that one or more constituents of an adequate diet, possibly protein, was responsible for this improvement. Protein may therefore be essential to liver-cell recovery. We have suggested that the clinical improvement which occurs within 48 to 72 hours of the institution of antibiotic therapy in patients with episodic stupor on a constant protein intake occurs because the production of toxic nitrogenous metabolites is prevented. An adequate diet which includes protein may therefore be given to the patient with hepatic coma within a short period of time after initiation of antibiotic therapy. Patients with hepatic coma at the Boston City Hospital are now managed according to the therapeutic regimen previously outlined,[7] except that protein in a restricted amount (30.0 to 40.0 gm. daily) is given 48 to 72 hours after commencement of antibiotic therapy.

Summary and Conclusions

The development of hepatic stupor with increased protein intake and its response to several antibiotics have been studied in two patients during repeated episodes of stupor and recovery. Arterial blood ammonia concentrations tended to parallel the clinical state, but the relationship was inconstant.

Nine patients with hepatic coma associated with hepatic failure and/or massive gastrointestinal hemorrhage were treated with a regimen designed to reduce the absorption of toxic nitrogenous metabolites. Seven patients improved, of which only three recovered.

The oral antibiotics used (especially neomycin and Paromomycin [Humycin]) were effective therapeutic agents for hepatic coma, unless some liver failure or massive uncontrolled gastrointestinal bleeding was underlying, when a fatal outcome was often the case. In cases of hepatic coma associated with hepatic failure antibiotics are recommended, and they allow the administration of protein, which may be essential to liver-cell recovery.

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Some Effects of Antibiotics on Nutrition in Man

Including Studies of the Bacterial Flora of the Feces

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In addition to the established efficacy of antibiotics in the prevention and treatment of diseases due to infectious agents, there are numerous observations which suggest that they may influence the nutrition of animals and man. Some reports indicate that certain antibiotics enhance the growth of several animal species and also of infants and children,[1] and although the mechanisms of this action are not clearly understood, the observed effects frequently have been attributed to an alteration of the bacterial flora of the gastrointestinal tract. Such alterations might influence nutrition by increasing or decreasing the quantities of certain nutrients available to the host through synthesis or utilization of vitamins and possibly of other essential nutrients by the bacteria in the intestinal tract. The possibility that certain antibiotics may influence nutrition by some direct effect on tissue metabolism must also be considered. Physical changes resembling those which occur in patients with vitamin deficiency have been noted in some patients treated with certain antibiotics.[2]

The studies reported here were designed to determine some of the metabolic effects of antibiotics administered to undernourished adult men and to evaluate the role of the gastrointestinal flora in human nutrition. They were carried out from December, 1950, through April, 1953, and preliminary reports of these investigations appeared in the Proceedings of the Annual Meetings of the American Society for Clinical Investigation in 1952 and 1953.[3,4]

Materials and Methods

Patients Studied.—Seven men were selected for study. Patient 3, age 49; Patient 6, age 37, and Patient 4, age 38, were chronic alcoholics who were admitted to the hospital because of minor respiratory infections which subsided with only symptomatic therapy. Patient 1, age 60; Patient 5, age 68; Patient 7, age 69, and Patient 2, age 64, had clinical scurvy on hospital admission, but this had been adequately treated several weeks prior to the institution of metabolic study; in Patient 2 studies were made on two separate occasions about a year apart. None of these seven patients displayed clinical evidence of vitamin deficiencies other than the ascorbic acid deficiency already indicated. All patients gave histories of inadequate dietary intakes with accompanying weight loss during the year prior to hospitalization and displayed physical evidence of undernutrition. This undernutrition was further confirmed by gains in body weight and the positive nitrogen balances accompanying the provision of adequate intakes of food. Thus, the patients selected were suitable subjects in whom to observe the influence of antibiotics upon body weight and nitrogen balance, since either favorable or adverse effects would be readily apparent.

None of these patients had clinical or laboratory evidence of liver, renal, cardiac, or other systemic disease at the time the studies were initiated, with the exception of Patient 7, who had 15% retention

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of sulfobromophthalein (Bromsulphalein) at 45 minutes after the intravenous injection of the dye.

Dietary Regimens.—The regular adequate hospital diet was prescribed for all patients for from one to four weeks before the metabolic studies were begun. This was intended to correct such vitamin-deficiency states as might exist but evade detection by clinical or laboratory methods. Furthermore, the patients consumed the food provided in the metabolic diets described below for approximately one week prior to the date from which the accumulation of data indicated in the figures and tables was begun. This was intended to assure a "steady state" prior to the institution of the study of each patient.

Throughout the studies the patients were maintained on a metabolic ward and were ambulatory. Each patient was given a constant diet, providing at least 75 gm. of protein and 2700 Cal. daily, except Patient 5, who weighed only 55 kg. and was maintained on 65 gm. of protein and 2000 Cal. Water was allowed ad lib., and daily outputs of urine were adequate. None of the studies was complicated by vomiting, although, because of anorexia, Patients 1 and 2 refused some of the food toward the end of the period of chlortetracycline administration. When this occurred, suitable replacements were provided to approximate the constancy of the basal diet. In no instance, however, does this interfere with the interpretation of the balance data, since the metabolic patterns resulting from the administration of chlortetracycline were initiated prior to the onset of anorexia and the necessity for food replacement. The volume and frequency of stools increased, and their character changed during antibiotic administration. Frank diarrhea did not occur during any of the studies. None of the patients developed any clinical evidence of specific nutritional deficiencies during these studies.

Nutritional Studies.—The patients were weighed at the same time each morning after voiding and defecating and prior to breakfast. Total daily urine volumes were collected and refrigerated in a brown-glass bottle with 10 ml. of a 5:1 mixture of glacial acetic acid and toluene as preservative. Care was taken to transfer promptly each urine specimen as it was obtained into the collecting bottle. Determinations of nitrogen in each day's urine were done by standard micro-Kjeldahl analysis. A suitable aliquot of each daily output of urine was then transferred to a small brown-glass bottle and stored in a Deepfreeze at —20 C for subsequent analyses as indicated below.

Stools were obtained in six- or nine-day pools during each study period. Each stool specimen was placed in a cardboard carton in the freezing compartment of a refrigerator immediately after it was obtained. Each day the stools were transferred in the frozen state to a large collection bottle and kept without preservative at —20 C. At suitable intervals throughout the studies freshly passed morning specimens of feces were weighed and appropriate small samples taken for bacteriologic study prior to freezing. In preparation for analysis each pooled specimen of feces was homogenized in a Waring Blendor to a measured final volume representing approximately a 1:5 dilution with distilled water. An aliquot of the total homogenate was taken for determination of nitrogen by macro-Kjeldahl analysis. A second aliquot, of approximately 250 ml., was centrifuged at 1500 rpm for 30 minutes to separate the sediment. The turbid supernate was decanted through gauze into a brown-glass bottle and stored at —20 C for microbiologic assays. The concentrations of the vitamins indicated in the text and tables were determined on appropriate dilutions of this "distilled-water extract" of pooled stools, and total daily outputs were calculated from the total volume of the homogenate. Attempts to quantitate the total vitamin content of stools following enzymatic (Taka-Diastase [powdered vegetable diastase] and papain) digestion of whole homogenates were not successful. The values for stool vitamin content, therefore, represent "free" or "available" microbiologic activity for the specific test organisms used.

The organisms and microbiologic methods used for the assay of urinary and fecal vitamin excretions include the following: Lactobacillus arabinosus 17-5 (ATCC 8014) for nicotinic acid (niacin) according to the method of Snell and Wright[6] as modified by Snell[8]; Saccharomyces carlsbergensis (ATCC 4228) for pyridoxine by the method of Atkin et al.[7] as modified by Rabinowitz and Snell[8]; L. casei (ATCC 7469) for folic acid by the method of Tepley and Elvehjem[9] and for riboflavin by the method of Snell and Strong.[10] Each vitamin was determined on daily aliquots of each urine specimen and on each pool of feces, except where otherwise indicated. Urine specimens were analyzed for amino acids by microbiological methods[11] and for N'-methylnicotinamide by the fluorometric method of Huff and Perlzweig.[12]

The plans of study are indicated for each subject. The effect upon body weight, nitrogen balance, and vitamin excretion was observed with the following antibiotics and daily dosages: chlortetracycline (Aureomycin) hydrochloride orally in doses ranging from 20 mg. to 3.0 gm., or a single intravenous dose of 1 gm.; oxytetracycline (Terramycin) hydrochloride capsules in doses of 2.5 gm.; bacitracin, 100,000 or 150,000 units, and polymyxin

B sulfate, 500 mg. by mouth.* The orally administered antibiotics were given in four divided doses distributed equally over the waking hours of the day. Periods of chlortetracycline administration in Patients 3, 2, and 1 were preceded and followed by the administration of placebos. The nitrogen content of these antibiotics was determined by macro-Kjeldahl analysis. Patient 5 received during one period of study "mild alkaline-rearranged compound A-377." This material consists of chlortetracycline which was so treated by the manufacturer as to destroy its antibacterial properties; the riboflavin given Patient 5 was purchased and provided in five equal daily doses.

Preliminary to the studies reported here, it was necessary to determine the possible influence of antibiotics contained in materials to be assayed by microbiologic techniques upon the results of the assay; these might be influenced either by inhibition of growth of the test organism by antibiotic contained in the material to be assayed or by replacement by antibiotic of a nutrient required for growth of the test organisms. Accordingly, studies were done to determine the sensitivity of microbiologic test organisms to the antibiotics used and the effect of those antibiotics on the growth responses of microbiologic test organisms to essential nutrients.

The details of these studies have been published [38] and provide validation for the microbiologic assay results included in this report. Since in the studies to be reported marked changes in urinary riboflavin excretion occurred following the administration of certain antibiotics, particular attention was given to the assay method for this vitamin. Furthermore, all microbiologic assays were done in triplicate or quadruplicate at different dilutions of unknown, and in no instance was any significant degree of inhibition or of enhancement of the growth responses of the test organisms apparent. These would be expected if the antibiotics or their degradation products contained in the samples of urine and feces were interfering in the assays.

Bacteriologic Studies.—Two or more specimens of feces from each period of observation in each patient were subjected to extensive bacteriological studies designed to give a more or less quantitative estimate of the salient changes in the fecal flora. The studies in the different patients were carried out by six different workers, and the methods were varied to some extent from patient to patient. In most instances simple and standard bacteriologic methods of counting and identification of the bacteria were used.

Gram-stained smears were made directly from the feces as soon as they were brought to the laboratory. Weighed amounts were diluted 1:10 or 1:100 in saline, homogenized, and used for quantitative counts, serial tenfold dilutions up to 10^{-10} being prepared in duplicate and used for pour plates or for streaking of a standard loopful on segments of appropriate solid media. These were incubated both aerobically and anaerobically. In addition, some of the homogenates were heated at 60 C for two hours before being diluted and cultured in or on the solid media. Blood agar, eosin-methylene blue agar, Sabouraud's agar, and thioglycollate broth were used for the cultures.

An ether-shake technique was also utilized to inhibit the growth of Gram-negative organisms and permitted the isolation of Gram-positive bacteria. Equal volumes of diluted stool and ether were shaken in a test tube for about 10 seconds. Pour plates or streaked plates were then prepared from serial dilutions of these suspensions. This method was of particular value in preventing overgrowth by Proteus.

In the last two patients, Patients 6 and 7, most of the specimens were also studied in parallel by quantitative techniques and with the use of differential media by Dr. W. P. Loh.[34] Some of his results are included and appropriately identified.

Assays for antibiotics were made of many of the specimens of feces obtained during and for a few days following cessation of administration of the antibiotics. The acid-acetone extraction method as described by McVay[35] was employed, and Streptococcus 98 was used as the assay organism for chlortetracycline. Assays for bacitracin and polymyxin were done on 1:1 mixtures of water suspensions and 95% ethanol and assayed with Streptococcus 98 and Escherichia coli (McLeod), respectively. The antibiotic sensitivity of selected isolates was tested by a twofold dilution method in appropriate broth or on agar plates.

Results

Although the general design of the study was the same for all of the patients, the

* The generic names chlortetracycline and oxytetracycline were introduced after these studies were completed. These terms appear in the text and tables, but the designations Aureomycin (trademark of Lederle Laboratories Division, American Cyanamid Company, for chlortetracycline), and Terramycin (trademark of Chas. Pfizer & Company, Inc., for oxytetracycline) are used in the figures. The chlortetracycline and alkaline rearranged Aureomycin were provided by Dr. Stanton M. Hardy, of the Lederle Laboratories Division, American Cyanamid Company; oxytetracycline, by Dr. Gladys L. Hobby, of Chas. Pfizer & Company; bacitracin, by Dr. Lawrence W. Smith, of Commercial Solvents Corporation, and polymyxin B sulfate (Aerosporin), by Dr. Donald F. Searle, of Burroughs Wellcome & Company, Inc.

number and types of observations varied in the different subjects. The results obtained in each patient will, therefore, be presented separately.

PATIENT 1.—This patient was maintained on a constant dietary intake containing 75 gm. of protein and a total of 2700±100 Cal. daily. Observations were made during a 9-day control period,† for 9 days while he was receiving 3.0 gm. of chlortetracycline daily, and during a final control period of 12 days.

Body Weight.—There was a steady gain in weight from 66.1 kg. to 69 kg. throughout the pretreatment period and the first three days of chlortetracycline administration. The weight then decreased steadily through the remainder of the period of antibiotic administration, after which it again increased.

Nitrogen.—Excretion of nitrogen in the urine remained fairly constant throughout the first control period and for the first six days of chlortetracycline; throughout this time the patient maintained a positive nitrogen balance ranging from +4 to +6.5 gm. (average, 5.2 gm.) daily. During the next four days the output of nitrogen in the urine increased, but there was still a slight positive balance; after that the pretreatment level of positive balance was reestablished.

Vitamins.—The daily outputs of urine were assayed for their content of riboflavin, pyridoxine, folic acid, leucovorin, and nicotinic acid. The results are presented in Table 1 as daily averages for three-day periods and for the total control and treatment periods. Fecal excretion of these vitamins, except for leucovorin (citrovorum factor), which was not done, was determined for each of the three periods. These results are presented in Table 7 and showed no marked differences related to antibiotic administration, except for possible decreases in excretion of pyridoxine and folic acid. The urinary excretion of riboflavin increased

† Some of the assays were omitted on the first three days of this period.

TABLE 1.—*Average Daily Excretion of Vitamins and Amino Acids in the Urine of Patient 1*

Period	Days of Observation	Riboflavin μg.	Pyridoxine, μg.	Folic Acid, μg.	Leucovorin μg.	Nicotinic Acid, Mg.	N'Methylnicotinamide, Mg.	Tryptophane, Mg.	Arginine, Mg.	Histidine, Mg.	Isoleucine, Mg.	Leucine, Mg.	Lysine, Mg.	Methionine, Mg.	Phenylalanine, Mg.	Threonine, Mg.	Valine, Mg.
I. Control, no CTC*	1–3	784	52	8.6	3.1	0.91	3.1	8.6	6.7	45.3	3.8	6.7	8.5	4.2	6.8	10.9	4.5
	4–6	421	50	10.8	2.8	1.02	2.7	8.6	9.4	35.5	3.9	7.6	5.5	3.5	5.7	9.7	4.8
	5–9	422	52	7.5	1.6	1.00	2.6	7.7									
	1–9	542	51	9.0	2.4	0.98	2.8	8.3	8.1	40.4	3.9	7.2	7.0	3.9	6.3	10.3	4.7
	4–9	422	51	9.2	2.0	1.01	2.6	8.1									
II. CTC, 3.0 Gm. daily	10–12	1144	55	7.0	2.0	0.90	3.7	7.5	9.3	56.4	2.6	7.4	6.4	3.4	5.9		4.9
	13–15	866	50	7.1	2.3	0.91	3.3	10.3	7.9	68.9	2.2	9.7		3.6	7.2	7.2	4.0
	16–18	1195	37	8.4	2.3	0.78	4.0	11.0	8.5	78.0	1.3	7.3	2.7	3.6	9.4	11.6	4.9
	10–18	1070	48	7.5	2.2	0.86	3.7	9.6	8.6	67.7	2.0	8.1	4.6	3.5	7.5	9.4	4.6
III. Control, no CTC	19–21	505	38	6.9	1.8	0.80	3.6	11.2	9.6	77.0	2.7	7.7	4.3	3.2	9.0	14.0	5.0
	22–24	221	37	4.5	2.0	0.80	2.7	9.4	9.0	57.7	4.5	8.5	6.8	5.5	8.4	14.2	5.8
	25–27	516	36	4.6	2.2	0.86	3.0	10.1	5.3	49.8	3.0	6.9	7.1	4.3	7.9	11.8	4.9
	28–30	505	46	4.2	1.9	0.77	2.6	8.0	10.3	54.9	2.9	7.6	6.2	3.8	6.7	12.4	5.0
	19–27	434	39	6.0	2.0	0.82	3.1	10.2	8.0	61.5	3.4	7.7	6.1	4.3	8.4	13.3	5.2
	19–30	452	37	5.6	2.0	0.81	3.0	9.7	8.6	59.9	3.3	7.7	6.1	4.2	8.0	13.1	5.2

* CTC indicates chlortetracycline orally.

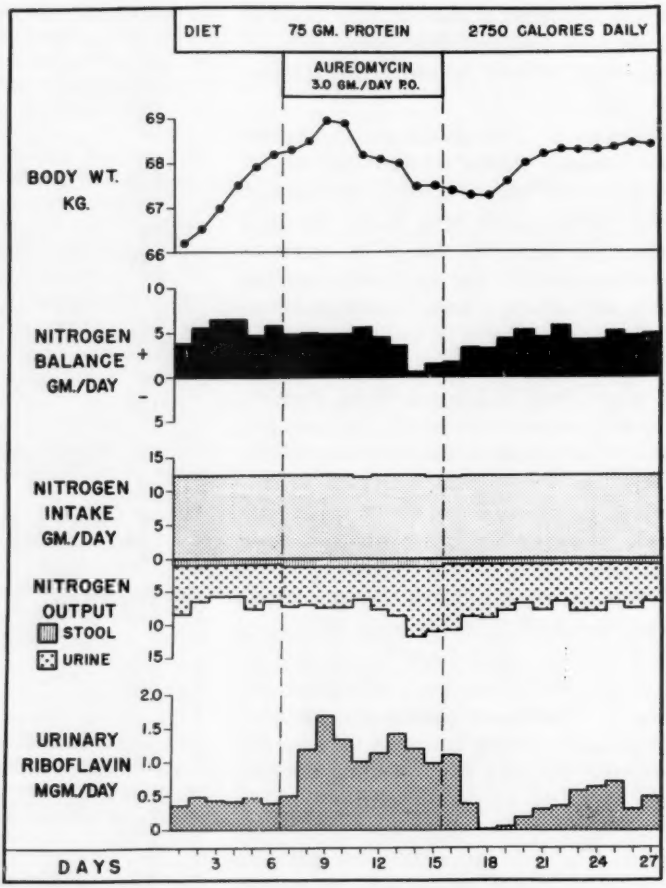

Fig. 1.—Effect of oral chlortetracycline (Aureomycin) on body weight, nitrogen balance, and urinary riboflavin excretion in Patient 1.

about two-and-one-half-fold during the antibiotic administration as compared with the control periods; this increased output began on the second day of treatment and ended on the second day after the antibiotic was stopped. The dietary intake of riboflavin was calculated to be about 2 mg. daily. Excretion of the other vitamins was not significantly altered during the chlortetracycline administration. However, although excretion of nicotinic acid was not altered, there was a suggestive increase in urinary excretion of the N′-methylnicotinamide in relation to the treatment (Table 1).

The daily observations on weight, nitrogen balance, and urinary riboflavin excretion in this patient are shown graphically in Figure 1.

Amino Acids.—Assays for the 10 "essential" amino acids were also done on each daily urinary output except the first three days of the initial control period. The average daily excretions of these substances are shown for three-day periods, for the entire treatment period, and for each of the control periods in Table 1. There was a definite increase in excretion of histidine during chlortetracycline treatment that continued through the first four days after it was stopped. Excretion of isoleucine was lower during the treatment period than it was before or in the subsequent control period. There was also a suggestive increase in excretion of tryptophane that began on the fourth day of treatment and continued into the post-treatment period.

Other Clinical Laboratory Data.—There was a transient increase in the blood hemoglobin (about 1 gm.) and hematocrit (5%) during chlortetracycline administration, with a return toward the original levels when treatment was stopped. The total leukocyte count was not significantly altered, but the total eosinophil count increased steadily from 110 (on four occasions) before treatment to 220 during the latter half of the treatment period and the succeeding week. Prothrombin time of the blood was unaltered. The urine was acid (pH 5.5±0.5) throughout the study, and its specific gravity ranged between 1.015 and 1.020. Urine urobilinogen excretion was only slightly reduced during the period of antibiotic administration (1:32 or 1:64 during both control periods and 1:8 or 1:6 during chlortetracycline treatment).

Character of Stools.—There were no important differences in the total weight and general character of the stools during the three periods of study except that those passed while the patient was taking chlortetracycline were greenish, somewhat more milky, and much less foul smelling than the others, which were light or dark brown.

Bacteriological Results.—Studies were made on seven specimens of feces during administration of chlortetracycline and four specimens obtained during each of the control periods.

Direct Morphology.—Gram-stained smears of the feces showed Gram-positive cocci and Gram-negative rods in all specimens. Gram-negative cocci (not identified) appeared transiently during treatment. Yeasts and fungous forms were each seen in a single specimen. Gram-positive bacilli appeared in the middle of the treatment period, increased in number, and persisted throughout the final control period.

Total Bacterial Counts.—There was a transient drop in the total number of bacteria counted in pour plates of serial dilutions of the feces during the first two days of treatment. After that, there was a steady increase during the entire period of observation, the counts reaching more than 10 times the pretreatment levels by the end of the period of chlortetracycline administration and 100 or more times higher in the post-treatment period. Both aerobes and anaerobes participated in these changes.

Coliforms.—E. coli was found consistently in all specimens and constituted an estimated 40% to 90% of the coliforms that were identified. Aerobacter aerogenes was first recognized on the sixth day of treatment and persisted during the rest of the treatment period. Proteus species were demonstrated only on the seventh day of treatment.

Gram-Positive Coccal Organisms.—Enterococci appeared for the first time on the last day of treatment and persisted through the post-treatment period. Hemolytic strains of pyogenes var. aureus (Staphylococcus) were present in two of the pretreatment specimens; they increased in number during chlortetracycline administration and persisted throughout the post-treatment period. All of these micrococci were coagulase-negative.

Anaerobes.—Clostridia were identified only on the last day of treatment and in the last two follow-up specimens. Gram-negative rods (Bacteroides) were obtained from anaerobic cultures of almost every specimen.

Susceptibility of the Fecal Organisms to Chlortetracycline.—Selected strains of coliform organisms and enterococci obtained at different times in relation to administration of the antibiotic were tested for sensitivity by the tube-dilution method. Of 31 strains tested, 18 were obtained before the end of the treatment period; these were all completely inhibited by 50μg. per milliliter or less. Of those isolated after the treatment ended, three strains each of Aerobacter aerogenes and E. coli were inhibited by 100μg. per milliliter and the other nine were sensitive to 50μg. or less per milliliter. There was, therefore, no marked change in the susceptibility of the coliform organisms to

chlortetracycline as a result of the treatment.

Summary.—In this patient, therefore, the administration of 3 gm. of chlortetracycline daily for nine days resulted in loss of weight and increased urinary excretion of nitrogen, accompanied by a marked increase in the urinary excretion of riboflavin. There were also some increases in urinary excretion of histidine and possibly also of N′-methylnicotinamide and tryptophane, and an apparent decrease in excretion of isoleucine related to taking the antibiotic. The bacteriologic data revealed no marked qualitative changes in the fecal flora, but there was a transient slight reduction followed by a marked increase in the total number of bacteria in the feces during the treatment, and this increased number persisted after the treatment was stopped.

PATIENT 2 (First Study).—This patient was studied on two separate occasions about a year apart. On the first occasion studies were made during nine days of administration of chlortetracycline orally, 2.5 gm. daily, and control observations were made for periods of nine days preceding and following this therapy. Throughout the 27 days the patient received a uniform diet containing 75 gm. of protein and 2700±100 Cal.

Body Weight.—The weight increased steadily from 43.8 to 46.1 kg. during the nine days before treatment was started and the first five days of chlortetracycline administration. This was followed by a similarly steady loss in weight to 44.7 kg. in the next seven days—through the third post-treatment day—after which an increase in weight was resumed.

Nitrogen.—The daily output of nitrogen into the urine during the first control period ranged from 5.3 to 8.7 gm. (average 6.6 gm.). This rate was maintained for the first four days of chlortetracycline administration, after which it increased to 12.7 gm. daily, the high values continuing through the second day after the antibiotic was stopped. The excretion of nitrogen in the urine then returned to pretreatment levels. Fecal nitrogen did not change significantly; it averaged 0.9, 0.8, and 0.7 gm. daily for the three successive periods of study. Nitrogen balance was positive (nearly +5 gm. daily) during the initial control

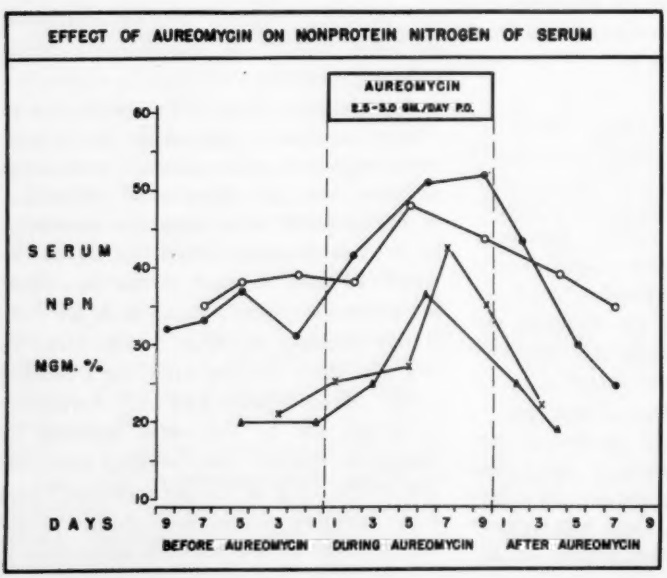

Fig. 2.—Increases of nonprotein concentrations in serum during oral administration of chlortetracycline. Open circles indicate the first study, in Patient 2; solid circles, the second study of this patient; triangles, Patient 3; crosses, Patient 4.

period and through the fourth day of antibiotic administration; this was reversed and the nitrogen balance was negative during the last three days of therapy. By the third post-treatment day a positive balance of about 5 gm. daily was restored.

The concentration of nonprotein nitrogen in the serum rose from 38 to 48 mg. per 100 ml. during chlortetracycline administration and returned to the original control values after the antibiotic treatment was stopped. (Open circles, Fig. 2).

Vitamins and Amino Acids.—Assays were done on each 24-hour specimen of urine for each of five vitamins and for tryptophane and N′-methylnicotinamide; other amino acids were assayed in pools of three-day collections of urine. The average daily urinary outputs of the vitamins and amino acids studied are listed in Table 2 for each three days and for each of the three study periods. Determinations on the feces were made only for riboflavin, nicotinic acid, pyridoxine, and folic acid; these were done on pools of feces for each of the three study periods, and the results are listed in Table 7.

Riboflavin excretion increased in the urine in relation to chlortetracycline administration beginning on the second day of this treatment and continuing through the second day after it was stopped. The rate of excretion then decreased to levels below those occurring during the initial control period and continued at the low levels throughout the final period of observation. The urinary excretion of pyridoxine, folic acid, leucovorin, and nicotinic acid did not show any significant change, but there was a suggestive increase in excretion of the N′-methylnicotinamide during the antibiotic administration. There was an apparent decrease in fecal excretion of riboflavin, pyridoxine, and folic acid in relation to the antibiotic administration, but nicotinic acid excretion was unchanged. The daily observations of the weights, nitrogen balance, and urinary riboflavin excretion are shown graphically in Figure 3.

TABLE 2.—*Average Daily Excretion of Vitamins and Amino Acids in Urine of Patient 2*

Period	Days of Observation	Riboflavin, Mg.	Pyridoxine, μg.	Folic Acid, μg.	Leucovorin, μg.	Nicotinic Acid, Mg.	N′Methylnicotinamide, Mg.	Tryptophane, Mg.	Arginine, Mg.	Histidine, Mg.	Isoleucine, Mg.	Leucine, Mg.	Lysine, Mg.	Methionine, Mg.	Phenylalnine, Mg.	Threonine, Mg.	Valine, Mg.
I	1–3	0.42	34	7.3	1.9	0.99	2.1	11.2	18.3	136	3.7	8.8	62	5.1	8.5	27	6.3
Control,	4–6	0.61	35	16.6	3.2	0.75	3.0	11.9	18.3	125	3.8	9.5	59	5.7	8.8	38	8.2
no CTC*	7–9	0.67	38	7.1	2.4	1.12	2.8	11.4	15.5	122	4.0	7.2	50	5.8	9.7	39	9.2
	1–9	0.56	35	10.3	2.5	0.95	2.6	11.5	17.4	128	3.8	8.5	57	5.5	9.0	35	7.9
II	10–12	1.16	39	6.4	1.9	0.93	3.6	13.4	12.6	152	3.1	7.6	39	3.6	10.5	21	9.5
CTC,	13–15	1.54	31	7.8	2.6	0.68	3.2	22.3	11.3	175	2.8	6.8	44	2.8	11.3	62	6.5
3.0 gm.	16–18	1.32	32	19.0	6.8	0.78	3.9	23.4	10.2	184	1.5	7.7	52	3.6	10.1	101	5.6
daily	10–18	1.36	34	11.1	3.8	0.80	3.6	19.7	11.4	170	2.5	7.4	45	3.3	10.6	61	7.2
III	19–21	0.77	35	11.7	3.7	0.86	4.9	17.7	16.4	170	3.1	9.1	83	5.2	10.4	93	7.7
Control,	22–24	0.18	29	3.1	1.4	0.61	1.3	10.8	12.2	96	3.2	7.4	47	4.0	6.8	35	6.8
no CTC	25–27	0.17	26	7.1	1.6	0.60	2.1	9.5	12.6	114	3.4	6.9	62	3.7	7.3	30	6.3
	19–27	0.37	31	7.3	2.2	0.69	2.8	12.7	13.7	127	3.2	7.8	64	4.3	8.2	52	6.9

* CTC indicates chlortetracycline orally.

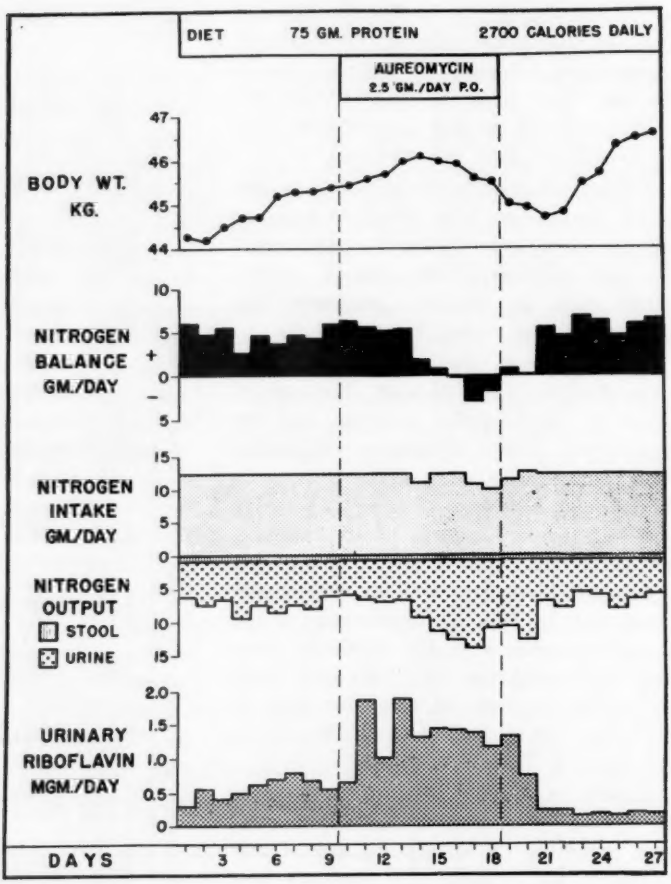

Fig. 3.—Effect of orally administered chlortetracycline on body weight, nitrogen balance, and urinary excretion of riboflavin in Patient 2 (first study).

The urinary excretion of tryptophane, histidine, and threonine increased beginning after the third day of the chlortetracycline period and continuing into the first three days after the antibiotic was stopped. There was no change in the urinary excretion of the other amino acids studied.

Other Clinical Laboratory Data.—Both the hemoglobin and the hematocrit rose slightly during the treatment period and then returned to nearly original levels by the end of the final period of observation. Urine urobilinogen was quantitated in two-hour samples daily; there was no significant change in relation to treatment.

Character of the Stools.—The daily output of feces varied only slightly in wet weight throughout the study; this averaged about 97, 115, and 128 gm. in the three successive periods. There was, however, a moderate increase in the bulk and fibrous content and a decrease in the fecal odor of the stools during and following the antibiotic administration.

Bacteriological Findings.—Various methods were used to detect gross qualitative and quantitative changes in the bacterial flora of the feces in relation to chlortetracycline administration. The results are summarized in Table 3 and show some irregular effects, depending on the method used.

Direct Morphology.—Gram-stained preparations of smears made of the fresh fecal

TABLE 3.—*Bacteriological Observations of Feces in Patient 2*

	Period of Study												
	I. Control			II. Oral Chlortetracycline, 3 Gm. Daily						III. Control			
Day of Observation	1	3	6	8	10	11	13	15	17	19	20	24	26
Direct Gram stain, estimated %													
Gram-positive bacilli	8	20	30	10	25	22	2	33	20	25	25	10	25
Gram-positive cocci	50	7	7	10	25	25	10	10	25	20	20	40	25
Gram-negative bacilli	4	60	60	80	50	50	85	50	50	50	50	50	50
Fusiforms and spirillae	2	10	0	0	0	0	0	5	1	1	1	<1	0
Yeasts	0	3	3	0	0	3	3	2	4	4	4	0	0
Bacterial counts, billions/gm.													
Total aerobes: pour plates	3	2	2	2	2	0.5	2	5	20	10	20	100	10
Dilutions streaked on blood agar	2	2	1	0.5	10	0.5	0.1	20	3	7	10	20	1
Dilutions streaked on EMB agar	1	1	2	1	1	0.5	3	1	7	2	1	1	2
Anaerobes on blood agar	3	1	1	7	2	1	4	1	9	4	1	10	3
Anaerobes on EMB agar	2	2	2	1	1	1	1	1	10	10	30	1	9
Aerobic growth (1:100 dilution) on blood agar	Good to heavy growth in every instance												
Types of colonies discerned	3	4	3	4	3	5	3	6	2	1	2	1	2
Distribution, %													
Coliforms	75	65	50	80	70	55	50	70	Ps *	Ps	Ps †	Ps	Ps †
Enterococci	25	30	45	20	25	40	50	25	--	--	--	--	--
Staphylococci	5	5	5	--	--	--	--	5	--	--	--	--	--
Others	--	0	0	--	5	5	--	--	--	--	--	--	--
Aerobic growth (1:100 dilution) on EMB agar	Good to heavy growth in every instance												
Types of colonies recognized	4	3	4	4	3	3	6	3	5	3	4	5	7
Estimated %													
Escherichia	60	15	60	40	98	80	10	0	5	25	35	20	15
Aerobacter	10	--	10	25	1	5	25	50	20	40	15	3	15
Pseudomonas	--	--	--	--	--	--	10	--	2	--	--	--	--
Proteus	<1	--	--	--	--	--	--	--	3	<1	10	2	5
Enterococcus	30	15	20	30	1	15	40	40	50	35	40	25	15
Others	--	70 ‡	10 ‡	5	--	--	15 †	10	20	--	--	50 ‡	50 §
Growth on blood agar after shaking with ether, estimated %	Growth slight to moderate												
Aerobes													
Streptococci ‖	100	100	¶	100	100	90	100 †	100 †	100	100	100	100	100
Staphylococci #	--	--		--	--	10	--	--	--	--	--	--	--
Anaerobic (number of types)	(2)	(2)		(3)	(3)	(3)	(3)	(4)	(4)	(4)	(3)	(2)	(1)
Streptococci	95	98		--	98 †	100 §	--	45	40	40	60	80 †	100
Staphylococci #	--	2		--	--	--	--	--	--	5	20	20	--
Clostridia	--	--		100 §	2	--	100 §	45 †	20	--	--	--	--
Others	5	--		--	--	--	--	10	40 †	15	20	--	--

* Proteus, swarmers. † 2 types. ‡ Pink, mucoid spreaders. § 3 types. ‖ Includes enterococci.
# All hemolytic and coagulose-negative. ¶ Unsatisfactory; coliforms not suppressed.

specimens showed no striking or sustained changes in distribution of morphological types of organisms, except for a transient decrease in the proportion of Gram-positive bacilli during the fourth and fifth days ‡ of antibiotic therapy.

Total Counts.—Pour plates of homogenized feces incubated aerobically showed a slight but transient decrease in the total number of aerobic bacteria on the second and third days of chlortetracycline administration. This was followed by a greater increase to tenfold over the control values by the end of the treatment period and still greater after treatment was stopped. The average numbers in the three successive periods were 1.5×10^9, 5×10^9, and 35×10^9.

Further quantitation was done by streaking a standard loopful of serial dilutions of the feces in duplicate on the surface of blood agar and of eosinmethylene blue agar and incubating each set aerobically and anaerobically; in addition colonies from the streaked plate of the initial (1:100) suspension were identified. Finally, the initial suspension was shaken thoroughly with ether in an attempt to eliminate the coliform organisms, and the proportion of aerobic and anaerobic organisms identified on surface growth was estimated.

Coliforms.—E. coli declined promptly in numbers and were almost entirely eliminated during treatment, returning to nearly the original numbers toward the end of the follow-up period. The numbers of Aerobacter decreased from the first through the third days of treatment and then increased. Proteus appeared on the last two days of chlortetracycline administration and persisted, tending to overgrow all other colonies on the surface cultures.

Gram-Positive Organisms.—Streptococci, predominantly enterococci with a varying proportion of α-hemolytic strains of a viridans Streptococcus, and clostridia were present during the first control period and persisted throughout the period of treatment. After the antibiotic was discontinued clostridia were no longer present in the cultures and staphylococci made up 5% to 10% of all the Gram-positive organisms; the latter were hemolytic and coagulase-negative.

Sensitivity of Fecal Organisms to Chlortetracycline.—Characteristic strains were isolated from each specimen and tested for susceptibility to chlortetracycline. Strains of E. coli isolated in both control periods were all inhibited by 6.25μg. to 25μg. per milliliter, but those isolated during administration of chlortetracycline required 100μg. per milliliter for complete inhibition. Strains of A. aerogenes isolated before treatment was started were inhibited by 25μg. or 50μg. per milliliter, those isolated during treatment required 100μg. per milliliter, and most of the post-treatment specimens were completely inhibited by 6.3μg. or 12.5μg. per milliliter. Strains of Proteus obtained on the last two days of treatment and later were resistant to, or only partially inhibited by, 200μg. per milliliter. Strains of Pseudomonas isolated during treatment varied in susceptibility, some were inhibited by 25μg. per milliliter, and others required 400μg. per milliliter to produce complete inhibition. Enterococci isolated in both control periods were all inhibited by 6.25μg. to 50μg. per milliliter, whereas those obtained from feces during treatment required 200μg. to produce complete inhibition. The staphylococci obtained after treatment was stopped were susceptible to 6.25μg. per milliliter.

Summary.—In this patient, maintained on a constant dietary intake of 75 gm. of protein and 2700 Cal., the oral administration of 2.5 gm. of chlortetracycline daily was associated with loss of weight, increased urinary excretion of nitrogen, and change from positive to negative nitrogen balance. There was also a marked increase in urinary excretion of riboflavin and some increase in the urinary excretion of tryptophane, histidine, and threonine. Fecal ex-

‡ Most of the observations were made daily in this patient, but some are omitted from the Table for the sake of brevity.

cretion of riboflavin, pyridoxine, and folic acid were decreased, and excretion of nicotinic acid was unchanged in relation to the chlortetracycline ingestion. Administration of the antibiotic was also associated with a brief and slight reduction in the total number of aerobic bacteria followed by a greater and more persistent increase in their numbers. E. coli was eliminated during treatment, and the number of A. aerogenes was temporarily reduced. Proteus and coagulase-negative Staphylococcus pyogenes var. aureus appeared, but clostridia could no longer be demonstrated after the treatment ended. The organisms isolated during chlortetracycline administration were less susceptible to that antibiotic than those isolated before or later.

PATIENT 3.—Observations in this patient were made over a period of nine days, during which he received 3.0 gm. of orally administered chlortetracycline daily, and during control periods before and after this treatment, when he was on an identical dietary regimen but received placebo capsules instead of antibiotic. A constant diet providing 2750 Cal. and 72 gm. of protein was maintained throughout the study. Fluid intake was kept constant at 2500 ml. daily.

Body Weight.—In this patient the weight remained stationary at about 71 kg. throughout the initial control period, rose gradually to 71.7 kg. by the sixth day of antibiotic administration, and then fell steadily during the next eight days to 70.2 kg. This was followed by a gradual rise, the original weight being regained by the end of the final period of study.

Total Nitrogen.—With a constant intake of 11.5 gm. of nitrogen daily, the output of nitrogen in the urine averaged 7.1 gm. daily during the initial control period; this increased steadily during the administration of chlortetracycline to a maximum of 13.5 gm. daily and then decreased to below pretreatment levels after the antibiotic was discontinued. Fecal content of nitrogen increased during treatment and averaged 0.5, 0.8, and 0.4 gm. daily for the three successive periods of study. The patient was therefore in positive nitrogen balance throughout the control periods and in negative balance during most of the period of chlortetracycline ingestion. The loss of nitrogen in the urine and feces of this patient was accompanied by a rise in concentration of nonprotein nitrogen in the serum from a level of 20 mg. per 100 ml. in the initial control period to 37 mg. per 100 ml. on the fourth day of treatment; this returned to pretreatment levels after the antibiotic was discontinued (Fig. 2).

Vitamins and Tryptophane.—In this patient quantitative assays were made of the daily urinary excretion of riboflavin, nicotinic acid, pyridoxine, folic acid, and citrovorum factor (leucovorin); fecal excretion of all but the latter were assayed in pools for the three periods of study. Among the amino acids, only the urinary excretion of tryptophane was determined. The average daily urinary excretion of these substances is listed for each three days and for each of the three periods of study in Table 4.

Riboflavin.—Intake of riboflavin was calculated to be 1.5 mg. daily. As in the previous patients, urinary excretion of this vitamin was fairly constant during the initial control period; this increased about two-and-one-half-fold during the oral administration of chlortetracycline and then dropped to levels below those found initially. Fecal recovery of riboflavin was also somewhat greater during the antibiotic administration in this patient; it averaged 0.46, 0.62, and 0.31 mg. daily in the three successive periods of study.

Pyridoxine.—Assays of the urinary and fecal excretion of this vitamin showed an enormous increase coincident with the administration of chlortetracycline. Assay of the antibiotic revealed the presence of pyridoxine, and it was then learned that the particular lot of chlortetracycline used in this patient contained 20 mg. per capsule, so that he was receiving 240 mg. of pyridoxine

TABLE 4.—*Daily Urinary Excretion of Certain Nutrients in Patient 3*

Period	Days of Observation	Volume of Urine, Ml./Day	Riboflavin, Mg.	Pyridoxine, μg.	Folic Acid μg.	Leucovorin, μg.	Nicotinic Acid, Mg.	Tryptophane, Mg.
I	1–3	1,990	0.74	55	2.9	1.25	0.94	18.3
Control, no CTC *	4–6	1,940	0.67	42	3.5	0.72	0.86	18.0
	5–9	2,130	0.58	51	5.8	0.57	0.88	16.6
	1–9	2,020	0.66	59	4.1	0.86	0.89	17.6
II	10–12	1,620	1.35	13,100	3.0	0.57	0.80	16.0
CTC, 3.0 gm.	13–15	1,920	1.37	16,000	3.6	0.89	0.72	21.1
daily †	16–18	1,920	2.18	18,900	4.9	0.92	1.01	15.9
	10–18	1,820	1.57	16,000	3.7	0.78	0.84	17.7
III	19–21	2,210	0.57	573	4.2	0.88	0.69	17.7
Control, no CTC	22–24	1,580	0.17	170	3.1	0.51	0.52	14.7
	25–27	1,130	0.18	80	3.0	0.45	0.49	14.4
	28–30	1,440	0.32	81	3.7	0.62	0.67	14.4
	19–30	1,590	0.31	226	3.4	0.67	0.59	15.3

* CTC indicates chlortetracycline orally.
† Contained 240 mg. of pyridoxine hydrochloride.

daily. However, by the method used, an average of only 16 mg. daily was assayed in the urine and 0.9 mg., in the feces during treatment. Increased urinary excretion as compared with pretreatment control levels continued throughout the 12 days of the final control period.

Fecal excretion of folic acid was decreased by about 50% during and after chlortetracycline administration. There was no change in urinary excretion of nicotinic acid, folic acid, and leucovorin or in the fecal excretion of niacin; the urinary excretion of tryptophane, likewise, showed no change in relation to the antibiotic therapy.

The daily measurements of weight, nitrogen exchange, and urinary riboflavin excretion are charted in Figure 4.

Other Clinical Laboratory Tests.—The following tests done on several occasions during each of the three periods of observation revealed no significant changes related to chlortetracycline administration: hemoglobin, red blood cell count, hematocrit, white blood cell count, urine specific gravity (1.015-1.020), pH of urine (approximately 5.5 throughout), and urine urobilinogen (1:16 or 1:32 in all tests). Serum bilirubin levels (direct, one minute) remained at levels of 0.03-0.06 throughout; however, by the indirect test there was a slight and gradual increase from an average pretreatment level of 0.12 to a peak level of 0.44 by the end of the treatment period, returning to 0.10 by the third day after the antibiotic administration was stopped.

Character of the Stools.—During the initial control period the patient had one formed stool daily; the average weight of the feces was 41 gm. daily. After the third day of treatment he passed two soft, bulky, semiformed stools daily, and the average wet weight of the feces for the entire treatment period was 123 gm. daily. During the post-treatment period of observations there was again one formed stool daily, and the average weight of the fecal output for the 12-day period was 64 gm. daily. During chlortetracycline administration the stools assumed a slightly greenish color and contained an increased amount of undigested food particles, and their odor became less fecal and more acrid.

Bacteriological Findings in Feces.—Studies of the stools were done on six specimens before treatment and on four specimens each during and after treatment. Each stool was examined by direct smear and Gram stain, pour-plate counts were made after incubating both aerobically and anaerobically with and without preliminary heating, and cultures were also made of dilutions in plain and thioglycollate both, as well as on surface plates.

Total Bacterial Counts.—Pour-plate counts of growth from serial tenfold dilutions of homogenized feces with and without prior heating at 60 C for 20 minutes

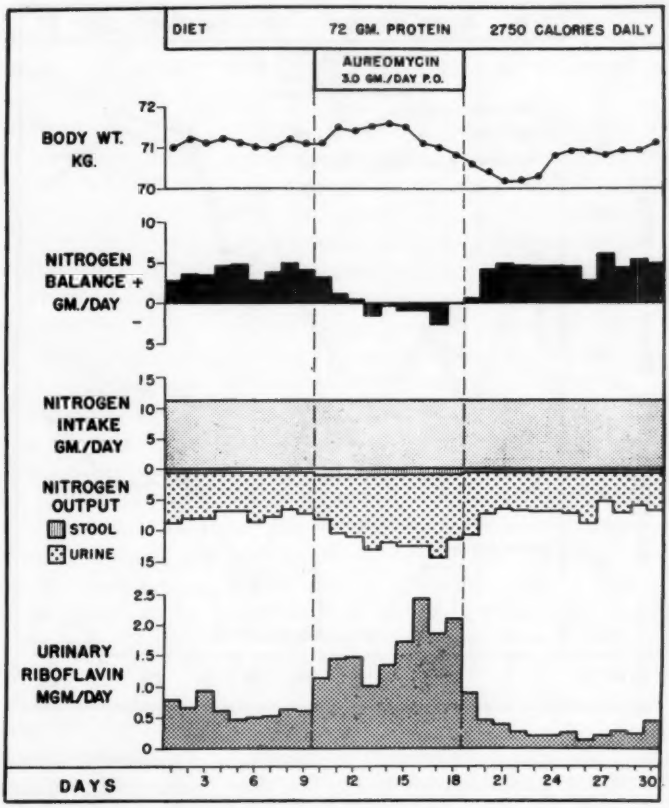

Fig. 4.—Observations on body weight, nitrogen balance, and urinary riboflavin in relation to administration of chlortetracycline orally in Patient 3.

were made with both aerobic and anaerobic incubation at 37 for 24 hours. The results are shown graphically in Figure 5. There was a slight decrease in the numbers of bacteria by all these methods during the first half of the treatment period, except that the decrease in the aerobic heat-resistant organisms (primarily enterococci) occurred only after the fifth day of chlortetracycline administration. The numbers of bacteria by each of the methods increased beyond the pretreatment levels either before or soon after the treatment ended.

Direct Smears.—Gram-stained smears of the feces were made each time and the relative proportion of the morphologic types was estimated. Gram-positive cocci constituted about one-fourth of all the organisms in the pretreatment period, this increased to nearly 50% or 60% during treatment but fell rapidly to below the original levels soon after treatment was ended. Gram-positive rods, which also constituted about one-fourth or slightly less of the organisms seen before the chlortetracycline was given, increased to about 40% during treatment, and this proportion persisted into the post-treatment period. Gram-negative bacilli constituted most of the remainder of the bacteria seen in the initial control period decreased to about 10% during treatment, but the original proportion was restored after the antibiotic was stopped. Only occasional yeast-like forms were seen before and after treatment.

Coliform Organisms.—Most of the drop in Gram-negative organisms was the result in the marked reduction in the number of Escherichia. However, A. aerogenes per-

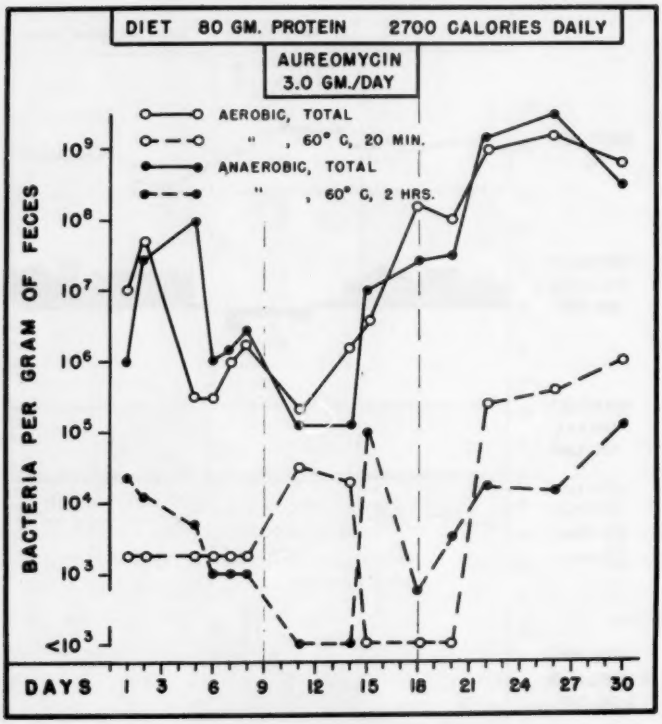

Fig. 5.—Effect of oral chlortetracycline on the numbers of bacteria in the feces of Patient 3.

sisted throughout treatment at approximately the initial control levels, but they increased about one-hundred-fold in the post-treatment period. Proteus organisms first appeared on the fourth day of treatment and persisted only through the second day after treatment was stopped.

Gram-Positive Organisms.—Hemolytic strains of Micrococcus pyogenes var. albus were made out only once in the pretreatment period and again on the fourth day of treatment and thereafter. These were all coagulase-negative. Enterococci were identified in only one specimen and α-hemolytic streptococci on two occasions before the chlortetracycline was started but in no others either during or after the treatment. Clostridia were present before antibiotic therapy; these were not made out in the cultures made of the first two specimens (second and fourth day) of chlortetracycline administration but then reappeared and persisted in all subsequent samples.

Sensitivity of Fecal Organisms to Chlortetracycline.—Coliform organisms isolated from surface cultures of pretreatment specimens were moderately sensitive to the antibiotic (all completely inhibited by 6.3μg. to 100μg. per milliliter. Strains of A. aerogenes obtained from feces during and after treatment all grew uninhibited in 100μg. per milliliter of chlortetracycline.

Levels of Chlortetracycline in Blood and Urine.—During treatment the levels of antibiotic in the serum done on several occasions ranged between 0.2μg. and 4μg. per milliliter, and the level in the urine was generally about 256μg. per milliliter. Four days after treatment was stopped, chlortetracycline no longer detectable in the serum, but a level of 4μg. per milliliter was still present in the urine; four days later the antibiotic was no longer detectable in the urine.

Summary.—In this patient, oral administration of 3 gm. of chlortetracycline daily

for nine days was associated with an increased urinary excretion of nitrogen, resulting in a negative nitrogen balance and a marked increase in urinary excretion of riboflavin occurred. It was also accompanied by a temporary reduction in the total number of organisms in the stools followed by an increase to levels higher than in the pretreatment control specimens. Strains of A. aerogenes that were relatively sensitive to chlortetracycline were replaced by more resistant ones during the treatment.

PATIENT 2 (Second Study).—The purpose of this study was to determine whether the biochemical and nutritional changes associated with chlortetracycline administration could be reproduced by nonabsorbable antibiotics which have similar or greater antibacterial action on the fecal bacteria. The combination of bacitracin and polymyxin B was used for this purpose. The patient was studied during five consecutive periods as follows: I, nine days without antibiotics; II, nine days of a combination of 100,000 units of bacitracin plus 500 mg. (500,000 units) of polymyxin B daily; III, eight days of the same amounts of these two antibiotics plus 2.5 gm. of chlortetracycline daily; IV, a repeat of II; and V, nine additional days without any antibiotics. The antibiotics were given in five equally divided doses every four hours during each day. The diet was kept constant throughout the study and contained 2800 Cal. including 80 gm. of protein daily. Fluid intake was not controlled. Each 24-hour output of urine was analyzed for total nitrogen and four vitamins, and three-day pooled specimens were assayed for 10 amino acids. The total fecal output for each of the five study periods was analyzed for total nitrogen and for the same four vitamins. Bacteriologic studies were made on feces on three to five days of each period.

Body Weight.—There was a steady gain of weight throughout the first two periods of the study from 44.6 to 48.3 kg. During the following period while chlortetracycline was being given and for the first two days after it was stopped, the weight dropped steadily to 47.0 kg., after which another rapid increase occurred and persisted throughout the remainder of the period of observation.

Total Nitrogen.—The daily excretion of nitrogen was relatively constant throughout the study except for the period beginning with the second day of chlortetracycline administration and ending one day after that antibiotic was stopped. During this period the daily urinary output of nitrogen increased about twofold. There was also a decrease in output before the initial average control values for nitrogen output in the urine were restored. The average daily amount of nitrogen increased in the feces during the period when the patient was taking bacitracin and polymyxin; but this was not further increased when chlortetracycline was added to this regimen. The patient was in negative nitrogen balance throughout all but the first day of the period of chlortetracycline administration and was in a net positive balance of nearly 5 gm. daily throughout most of the other days of the study.

The level of nonprotein nitrogen in the serum, which was 35 ± 3 mg. per 100 ml. during the first two periods of study, increased steadily to 52 mg. per 100 ml. during the eight days of chlortetracycline administration and decreased below 30 mg. per 100 ml. after that antibiotic was stopped (Fig. 2, solid circles).

Vitamins.—Urinary excretion of riboflavin was not affected by the ingestion of bacitracin and polymyxin; it was increased only when chlortetracycline was also given and to about the same degrees as in the previous experiments. Recovery of riboflavin in the feces was somewhat less during chlortetracycline ingestion and still lower in the following period than at other times. For the five successive periods the average daily amounts of riboflavin found in the feces were 1.56, 1.04, 0.72, 0.52, and 0.84 mg.

The urinary excretion of folic acid was increased during the chlortetracycline period,

TABLE 5.—*Average Daily Output of Certain Vitamins, Amino Acids, and Other Substances in Urine of Patient 2 (Second Study)*

Period *	Days of Observation	Urine Volume, Ml.	Total Nitrogen, Mg.	Riboflavin, Mg.	Pyridoxine, μg.	Folic Acid, μg.	Nicotinic Acid, Mg.	Tryptophane, Mg.	Arginine, Mg.	Histidine, Mg.	Isoleucine, Mg.	Leucine, Mg.	Lysine, Mg.	Methionine, Mg.	Phenylalanine, Mg.	Threonine, Mg.	Valine, Mg.
I	1-3	1,990	6.5	0.52	41	1.9	0.46	9.5	4.2	95	3.9	6.0	34	2.9	7.8	18.5	3.4
Control,	4-6	2,070	6.6	0.51	38	1.7	0.55	9.5	8.7	104	5.2	7.9	28	2.0	8.9	19.2	5.6
no anti-	7-9	2,060	7.1	0.53	42	3.6	0.55	11.5	7.2	117	4.4	8.7	35	2.8	9.5	24.5	6.6
biotic	1-9	2,040	6.7	0.52	40	2.4	0.52	10.2	6.7	105	4.5	7.5	32	2.5	8.7	20.7	5.2
II	10-12	2,150	7.1	0.60	46	3.3	0.55	11.7	4.5	110	2.1	6.2	18	3.3	9.4	21.7	3.2
B+P	13-15	1,480	7.0	0.64	51	3.3	0.50	10.5	4.6	105	2.0	6.4	15	2.0	8.9	18.8	2.8
	16-18	2,050	7.4	0.66	51	3.7	0.54	13.2	7.0	105	3.3	7.8	13	3.0	10.5	20.5	4.1
	10-18	1,890	7.2	0.63	49	3.5	0.53	11.8	5.3	106	2.5	6.8	15	2.8	9.6	20.3	3.4
III	19-21	1,750	10.4	1.72	49	3.6	0.67	14.4	5.6	132	2.1	--	21	1.4	9.3	14.5	1.9
B+P	22-24	1,390	12.5	1.30	45	6.9	0.65	22.7	4.2	96	--	6.1	29	1.1	8.6	63.1	--
+ CTC	25-26	1,610	14.5	1.33	52	8.0	0.88	27.5	6.6	206	--	5.3	52	4.1	11.6	113.2	--
	19-26	1,580	12.5	1.47	48	5.8	0.69	20.8	5.3	137	2.1	5.8	32	2.0	9.6	57.4	1.9
IV	27-29	1,210	10.9	0.66	41	4.3	0.59	18.4	10.4	187	2.1	7.7	39	7.8	9.4	99.8	4.1
B+P	30-32	1,180	6.3	0.28	32	2.9	0.45	12.6	8.9	124	2.2	4.9	35	2.5	5.9	54.5	3.3
	33-35	1,330	6.3	0.35	44	2.7	0.57	15.2	13.3	137	6.3	10.8	30	3.9	9.7	40.2	5.2
	27-35	1,240	6.8	0.43	38	3.4	0.54	15.4	10.9	149	3.5	7.8	34	4.7	8.3	64.8	4.3
V	36-38	1,050	6.6	0.47	44	3.9	0.52	17.3	6.8	151	3.4	7.5	48	4.3	10.2	48.7	6.2
Control,	39-41	1,000	6.7	0.83	44	3.7	0.66	18.6	4.5	104	--	--	20	3.0	10.5	34.7	7.6
no anti-	42-44	825	6.8	0.60	40	2.9	0.72	19.6	17.8	159	5.6	10.9	36	7.5	11.5	44.0	10.2
biotic	36-44	960	6.7	0.74	42	3.4	0.63	18.5	9.7	138	4.5	6.1	34	4.9	10.7	42.5	8.0

* B indicates bacitracin, 100,000 units daily; P, polymyxin B, 500 mg. daily; CTC, chlortetracycline, 2.5 gm. daily; all antibiotics given orally.

but there was no discernible effect of any of the antibiotic treatments on the excretion of nicotinic acid and pyridoxine (Table 5). However, the amount of these three vitamins determined in the feces was considerably lower during the period when chlortetracycline was given than in the other four periods; this was most striking in the case of pyridoxine (Table 7).

The daily measurements of body weight, total nitrogen exchange, and urinary riboflavin excretion are shown graphically in Figure 6.

Amino Acids.—The daily urinary excretion of tryptophane, histidine, and threonine increased somewhat during chlortetracycline administration as compared with the two preceding periods, and the increases persisted to some extent through the remainder of the study (Table 5).

Other Clinical Laboratory Findings.—The hemoglobin level increased steadily throughout the study and showed a net gain of about 1 gm.; this was accompanied by an increase of 500,000 red blood cells per cubic millimeter. The hematocrit rose from 40 to 45 by the end of Period III and subsequently dropped to the original control levels. The total and differential white blood cell counts showed no change. The direct

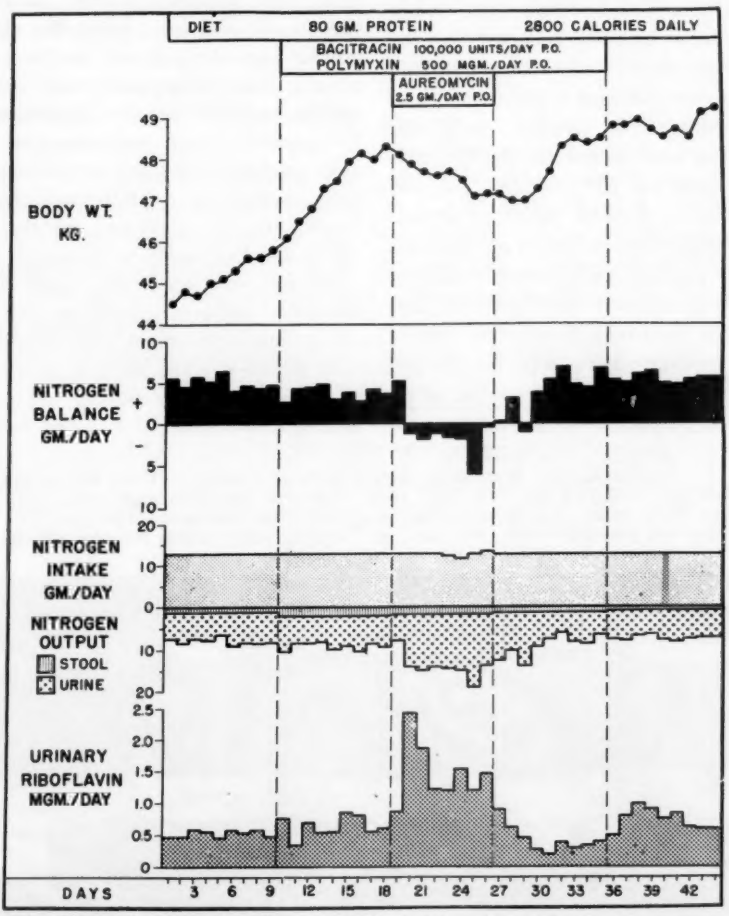

Fig. 6.—Observations on the effects of oral administration of bacitracin plus polymyxin with or without chlortetracycline on body weight, nitrogen balance, and urinary excretion of riboflavin in second study of Patient 2.

(1 minute) serum bilirubin level remained at or below 0.1, but the 15-minute readings increased from an average 0.24±0.3 in the first two periods to 0.54 during the time when chlortetracycline was added and then returned to the original control levels in the final two periods of study, but no changes occurred in urine urobilinogen.

Character of the Stools.—The weight and bulk of the stools nearly doubled after the antibiotics were started and did not return to the pretreatment state until all antibiotics were discontinued. The total output for the consecutive periods are shown from left to right in Figure 7; each of the jars contains the output for a period of nine days, except the middle one, which was collected over the period of eight days of administration of all three antibiotics—bacitracin, polymyxin, and chlortetracycline. The average daily wet weight of the fecal output for the five consecutive periods was 109, 200, 183, 156, and 110 gm. During each of the three periods when antibiotics were being given, the feces were softer and less well formed, contained much more undigested food particles, and had less of a fecal odor than in the control period. However, there was no marked difference in the appearance of the stools when chlortetracycline was added as compared with the periods when only bacitracin and polymyxin were given.

Bacteriological Observations on Fecal Flora.—Direct Smear: The proportions of the different morphologic types of bacteria were estimated from Gram-stained smears of fresh feces. In the initial control period, about 60% of the bacteria seen in such smears were Gram-negative, mostly bacilli; the rest were Gram-positive organisms, about two-thirds of them being bacillary forms and the others, cocci in pairs or short chains. Only occasional yeast forms were seen. After treatment with bacitracin and polymyxin was started, the Gram-positive forms decreased rapidly, and only rare ones were seen until after the chlortetracycline was stopped; at that time, Gram-positive rods reappeared and constituted nearly one-half of the organisms seen. Gram-positive cocci reappeared in appreciable numbers only after all antibiotics were stopped and soon constituted about one-fourth of the organisms in the smears. Gram-negative bacilli were almost the only organisms seen from the fourth day after bacitracin and polymyxin were started until all antibiotic treatment was stopped; there was, however, some increase in the number of yeast-like forms during the period of antibiotic administration. The latter were

Fig. 7.—Total collections of feces in second study of Patient 2. From left to right: I, Days 1-9, diet alone, 978 gm.; II, Days 10-18, bacitracin plus polymyxin orally, 1787 gm.; III, Days 19-26, bacitracin plus polymyxin plus chlortetracycline orally, 1460 gm.; IV, Days 27-35, bacitracin plus polymyxin, 1403 gm.; V, Days 36-44, diet alone, 992 gm. (Note: each period was nine days except III, which was eight days.)

only rarely seen in the final control period when the Gram-positive and Gram-negative bacterial forms returned to about their original pretreatment proportions.

Total Bacterial Counts (Fig. 8).—The total numbers of aerobic and anaerobic bacteria, as determined by pour plates of serial dilutions of homogenized fecal suspensions, declined somewhat soon after the oral bacitracin and polymyxin were started but returned to control levels during the period when chlortetracycline was also given and did not increase further. Heat-resistant aerobes (enterococci) and anaerobes (clostridia) were practically eliminated during Period II, when bacitracin and polymyxin were given and did not return in appreciable numbers until all antibiotics were discontinued; they then reappeared in the same, or slightly greater, numbers than in the initial pretreatment period.

Coliforms.—E. coli and A. aerogenes predominated in pretreatment stools. The former were not identified throughout the three periods of antibiotic administration, and the latter was gradually eliminated after treatment with bacitracin and polymyxin were started. Both were again present after all antibiotics were stopped. Proteus organisms first appeared during the fifth day after bacitracin and polymyxin were started, and they persisted throughout the rest of the study.

Yeasts and fungi were grown in each period but they (predominantly Candida) were most numerous during the periods when antibiotics were being given.

Susceptibility of the Fecal Organisms to the Antibiotics Used.—All of the strains of the various forms of bacteria isolated during any of the periods when the antibiotics were being given were resistant to 50 units of bacitracin, 50μg. of polymyxin, and 100μg. of chlortetracycline.

Recovery of Antibiotics in the Stools.—The feces, diluted 1:10, were kept in the frozen state at −20 C and then were thawed, further diluted in distilled water, and passed through a Seitz filter before they were assayed for their content of antibiotics by a serial dilution method. Chlortetracycline could not be detected in any of the stools by this method. About 2.5% to 13.5% of the administered bacitracin and 5% to 20% of the amount of polymyxin ingested was found in the feces.

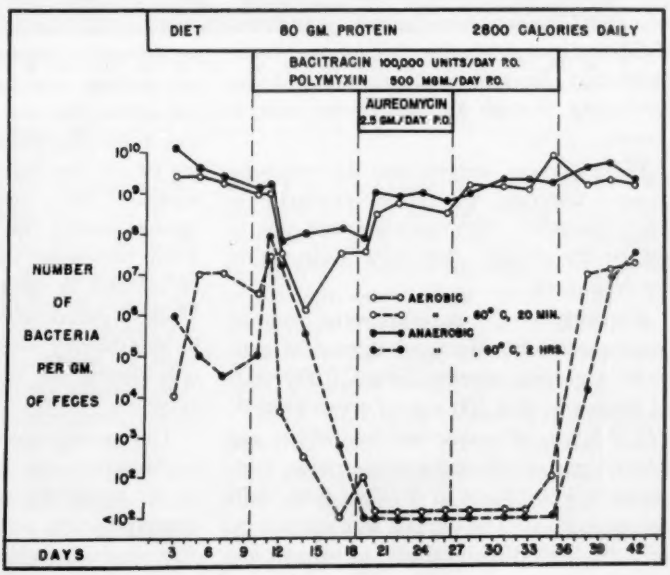

Fig. 8.—Effect of oral administration of bacitracin plus polymyxin with and without chlortetracycline on the numbers of bacteria in Patient 2, second study.

Summary.—In this patient, oral administration of bacitracin plus polymyxin, with or without chlortetracycline, resulted in an increase in the bulk of the stools; this was accompanied by reductions in bacterial counts that were greater than in the previous study in this patient, when chlortetracycline alone was given. The reduction in bacterial counts in the present study was not followed by an increase above original control levels, as it was in the earlier one. The reduction in the bacterial counts was due largely to the elimination of E. coli, enterococci, and clostridia. Administration of bacitracin and polymyxin by mouth did not produce any significant changes in excretion of any of the nutritional substances that were studied and did not alter the gain in weight or positive nitrogen balance during a dietary regimen that was constant in protein and calories. The addition of chlortetracycline to the same regimen was associated with a loss of weight, negative nitrogen balance due primarily to increased urinary excretion of nitrogen, slight elevation of nonprotein nitrogen in the serum, increased excretion of riboflavin in the urine, and decreased fecal excretion of nicotinic acid, pyridoxine, and possibly folic acid. There were also increases in urinary excretion of the amino acids tryptophane, histidine, and threonine, beginning at the time when chlortetracycline was started and continuing through the subsequent control period.

These findings suggest that the metabolic changes observed are related primarily to chlortetracycline administration and not to any of the changes that were made out in the fecal flora.

PATIENT 4.—Comparisons were made of some nutritional effects of three oral antibiotic regimens, namely, (1) 150,000 units of bacitracin plus 500 mg. of polymyxin B; (2) 2.5 gm. of oxytetracycline alone, and (3) 2.5 gm. of chlortetracycline alone; these amounts were given in divided doses daily for nine days. The patient was maintained on a constant diet of 80 gm. of protein and 3400 Cal. throughout the study, and control periods on this diet alone preceded and followed each period of antibiotic administration. Daily observations were made on body weight, urinary and fecal output of nitrogen, and urinary excretion of riboflavin. Assays of the pyridoxine and nicotinic acid excreted in the urine were done for the last three days of each period of study. Fecal content of nitrogen and of these three vitamins and folic acid were quantitated in the pooled total output of each period. Bacteriologic studies were done on three or more stools collected in each period and on the last day of observation.

Body Weight, Nitrogen Balance, and Urinary Riboflavin (Fig. 9).—There was a more or less steady increase in body weight throughout the study, interrupted only by a slight drop during five of the days of chlortetracycline administration. Urinary excretion of nitrogen increased during oxytetracycline and markedly during chlortetracycline administration and was quite constant throughout the other periods of study. Fecal excretion of nitrogen was increased over that of control periods during the ingestion of bacitracin plus polymyxin and of chlortetracycline but not while oxytetracycline was given. There was a negative nitrogen balance during the oxytetracycline period and a greater negative balance while the patient was taking chlortetracycline; throughout the rest of the study the patient was in positive nitrogen balance, except during two of the days on bacitracin plus polymyxin. The smaller positive nitrogen balance during the administration of bacitracin plus polymyxin was due primarily to an increase in the fecal output of nitrogen. Urinary excretion of riboflavin appeared to be significantly increased over control levels only during the administration of chlortetracycline.

The concentrations of nonprotein nitrogen in the serum rose from control values of 20 to 22 to 32 mg. per 100 ml. during the oxytetracycline period and to 32.4 mg. per 100 ml. during chlortetracycline administra-

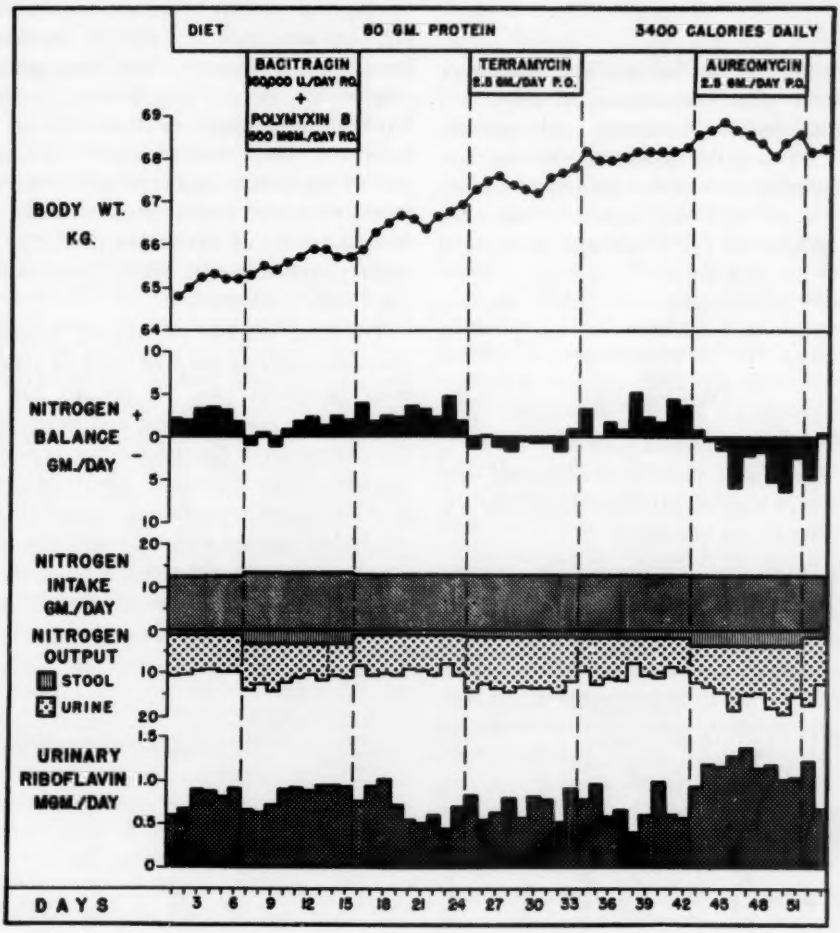

Fig. 9.—Observations on the effects of three oral antibiotic regimens on body weight, nitrogen balance, and urinary excretion of riboflavin in Patient 4.

tion, returning rapidly to control levels after these antibiotics were stopped.

Other Vitamins.—The data for the average daily exchange of total nitrogen and for the average daily excretion of four vitamins, including riboflavin, in this patient are given in Table 7. Fecal excretion of riboflavin was somewhat greater during each of the periods of antibiotic administration as compared with those when no antibiotics were given; however, it was greatly increased only during the chlortetracycline period. No clearly defined effect of administration of any of the antibiotics was discerned on the urinary or fecal excretion of nicotinic acid or on the urinary excretion of pyridoxine. Fecal excretion of the latter varied widely but not consistently in relation to the antibiotics. Fecal excretion of folic acid appeared to be somewhat greater during each of the antibiotic periods as compared with that in the control periods.

Other Clinical Laboratory Findings.—Based on tests done on two or more occasions during each period of the study there were no changes in hemoglobin, red blood cell count, total and differential white blood cell counts, and urinary urobilinogen. The

pH of the urine in all specimens was about pH 5.5.

Character of the Stools.—The stools were definitely softer, were greater in weight and bulk, had less of a fecal odor, and contained larger amounts of undigested fibrous material during each of the periods of administration of antibiotic, especially bacitracin plus polymyxin. The average daily fecal output was as follows:

Period	Antibiotics	Gm./Day
I	None	109
II	Bacitracin+polymyxin	380
III	None	164
IV	Oxytetracycline	234
V	None	222
VI	Chlortetracycline	292

The stools were liquid and unformed during one 24-hour period while the patient was receiving oxytetracycline.

Bacteriological Findings.—The relevant quantitative findings in the bacterial flora of the feces in relation to the various antibiotic regimens are listed in Table 6. In stained smears made directly from homogenized suspensions of 1:100 dilutions of feces, Gram-negative bacilli predominated in all specimens except those obtained in the latter half of the bacitracin plus polymyxin period, when yeast-like forms predominated, and toward the end of and immediately after the oxytetracycline period, when Gram-positive organisms predominated.

The total number of bacteria decreased transiently during the first days of therapy with bacitracin plus polymyxin, but decreased to a greater extent and remained at the lowest level throughout the period of oxytetracycline ingestion. The total counts rose above pretreatment control levels before the end of therapy with bacitracin plus polymyxin and with chlortetracycline and increased steadily and to the greatest extent in

TABLE 6.—*Observations on Fecal Flora in Patient 4*

Period *	Days of Observation	Gram-Stained Smears, Extimated %				Pour-Plate Counts					
		Gram-Pos. Cocci	Bacilli		Yeasts	Total Aerobes, $\times 10^6$	Gram-Positive Cocci, $\times 10^5$ †	Coliform, $\times 10^6$	Yeasts $\times 10^5$	Proteus, $\times 10^7$	Antibiotics /Gm. of Feces §
			Gram-Pos.	Gram-Neg.							
I	1	13	10	76	1	51.	0	51A	0	0	
Control	3	--	5	95	--	3.	0	3A	0	0	
	6	10	25	65	--	1.4	0	0.4A	0	0	
	8	--	5	92	3	0.1	0.4S	0	1	0	P, 0.2;B, 400
II	9	3	--	90	7	10.	0	0	100	0	P, ?; B, 240
B + P	11 ‖	--	--	40	60	170.	0	0.004A	1700	0	P, 1.3;B, 400
	14	2	--	38	60	440.	0	390A	500	0	P, 0.2;B, 100
	16	3	--	92	5	310	0	280A	300	0	
III	21	--	10	89	1	115	550En	60A+E	0	0	
Control	23	15	5	77	3	15	27En	12A+E	0	0	
	25	38	7	55	--	1.3	0	1.3A+E	0	0	
IV	27	--	--	100	--	0.01	0	0.01A	0	0	OCT, 0.25
OTC	29	--	63	33	2	0.01	0.02S	0	0.05	0	
	32	--	6	90	4	0.03	0.34S	0	0	0	
	34	60	7	32	--	00.23	2.3S	0	0	0	
V	36	16	2	82	--	850.	0	850A	0	0	
Control	39	11	9	73	7	1,000.	0	1,000A	0	0	
	42	7	8	84	1	28,500.	0.1S	28,500A	0	0	0
	43	2	11	81	6	610	6100En +0.06S	0.5A	0	0	CTC, 0.06
	45	3	14	77	6	0.04	0.5S	0	0	0	
VI	47	5	11	79	5	30	2.5S	0	0	3	
CTC	49	3	5	89	3	5,100	3.7S	0	0	5,100	
	51	7	2	90	1	1,000	2.7S	0	0	1,000	CTC, 0.25
VII Control	53	11	5	81	3	815	1.6S	15A	0	80	CTC, 0.03

* B indicates bacitracin, 150,000 units; P, polymyxin, 500 mg.; OCT, oxytetracycline, 2.5 gm.; CTC, chlortetracycline, 2.5 gm.; all given orally in divided doses daily.

† S indicates staphylococci; En, enterococci; on day 44 and thereafter, special staphylococcal medium used (data from Dr. Loh).

‡ A indicates aerobacter; E, escherichia; with use of other methods, Dr. Loh studied the specimens of Days 44 and thereafter and reported the following numbers of coliforms: 6.9×10^8, 3.8×10^8, 5×10^5, 6×10^4, 2.3×10^8, 2×10^7, and 3.6×10^7 on Days 44, 45, 47, 49, 51, 53, and 55, respectively.

§ Concentration in units of bacitracin and mg. of other antibiotics.

‖ Very few organisms seen in direct smears.

the period following oxytetracycline administration.

Enterococci, in moderate numbers, were observed only during the end of control Period III and immediately after control Period V. Micrococci were first encountered after the middle of the oxytetracycline period; they were not found in the middle of the following control period but were observed in moderate numbers in all subsequent specimens. Coliforms were markedly reduced and not identified during the first half of the bacitracin-polymyxin period and after the second or third days of treatment with each of the tetracyclines.§ Proteus organisms first appeared on the fifth day of chlortetracycline therapy and were present in moderately large numbers in all subsequent specimens. Most striking was the relatively large number of yeasts (Candida) grown from the feces during and immediately after treatment with bacitracin plus polymyxin. Although yeast-like forms were seen in direct smears of most of the other specimens, they could subsequently be grown on only a single occasion—during oxytetracycline therapy.

Concentration of Antibiotics in Feces.—Several of the fecal specimens were assayed for their content of antibiotics; the results are listed in the last column of Table 6. Concentrations of 100 to 400 units of bacitracin and 0.2 to 1.3 mg. of bacitracin per gram of feces were found during treatment with this pair of agents. Concentration of the tetracyclines ranged from 60μg. to 250μg. per gram of feces during administration of these antibiotics and 30μg. per gram of chlortetracycline were still present in the feces on the second day after administration of this antibiotic was stopped.

§ The specimen obtained immediately before chlortetracycline administration was started and all subsequent ones were also studied quantitatively by Dr. Loh,[34] with use of methods designed specifically to identify micrococci and coliforms. His studies indicated that staphylococci and the coliforms persisted throughout the chlortetracycline period with only a temporary one-thousand-fold reduction in the numbers of the latter during the third day of that treatment.

Antibiotic Sensitivity of Fecal Organisms.

The strains of Micrococcus pyogenes var. aureus isolated early in the bacitracin-polymyxin period grew in 5 units of bacitracin and were completely inhibited by 50 units of bacitracin and by 6.25μg. per milliliter of oxytetracycline and chlortetracycline; those isolated during therapy with the tetracycline required 400μg. and 200μg. per milliliter of oxytetracycline and chlortetracycline, respectively, to produce complete inhibition. The Candida isolated during the bacitracin-polymyxin period and again during oxytetracycline therapy and the Proteus organisms isolated when the patient was receiving chlortetracycline were all resistant to 500μg. per milliliter of the two tetracycline antibiotics and polymyxin and to 500 units of bacitracin.

Summary.—In Patient 4, oral administration of bacitracin plus polymyxin had very little effect on the total nitrogen exchange, whereas 2.5 gm. daily of either oxytetracycline or chlortetracycline by the same route changed a considerable positive nitrogen balance into a negative nitrogen balance, which was moderate in the case of the former and marked during administration of the latter. Riboflavin excretion was significantly altered only during the ingestion of chlortetracycline, when both the urinary and fecal excretion of this vitamin was increased over control levels. During each of the antibiotic periods the bulk of the feces increased and there was a reduction in the total number of bacteria, which was temporary, except during the oxytetracycline period, when it persisted throughout the treatment. In each instance the lowered bacterial count was followed by a greater increase above control levels, the greatest increase occurring after oxytetracycline administration was stopped. Coliform organisms were markedly reduced or eliminated during each of the antibiotic regimens. Candida in moderate numbers were grown only during and immediately after treatment with bacitracin plus polymyxin, and Proteus organisms appeared during chlortetracycline administration and

persisted. The organisms isolated from feces during antibiotic administration were resistant to the antibiotics used in this study.

PATIENT 5.—This patient, who weighed about 56 kg. at the start, was studied over a period of nearly five months, during which he was maintained on a constant diet that provided 65 gm. of protein and 2000 Cal. daily. During the entire time, observations were made on body weight, nitrogen balance, urinary riboflavin excretion, and fecal bacteriology. Additional studies on excretion of pyridoxine, nicotinic acid, folic acid, and some amino acids were also done during part of the time. Various regimens were instituted for periods of nine or more days, preceded and followed by suitable control periods. The studies in this patient were designed (1) to determine whether the nutritional changes observed during oral administration of chlortetracycline were dependent on the antibacterial activity of this agent, (2) to restudy and compare the nutritional effects of oxytetracycline and chlortetracycline in the same oral doses, (3) to examine the effect of oral administration of riboflavin on the changes in nitrogen balance produced by oral chlortetracycline, and (4) to determine the effects of a small oral dose (150 mg. daily) of chlortetracycline as compared with the usual dose of 2 to 3 gm. daily.

Body Weight, Nitrogen Balance, and Urinary Riboflavin Excretion.—The regimens instituted and the daily observations on body weight, nitrogen balance, serum nonprotein nitrogen concentrations and urinary excretion of riboflavin during the first 100 days of the study are shown in Figure 10. Diet alone was adequate in this patient to pro-

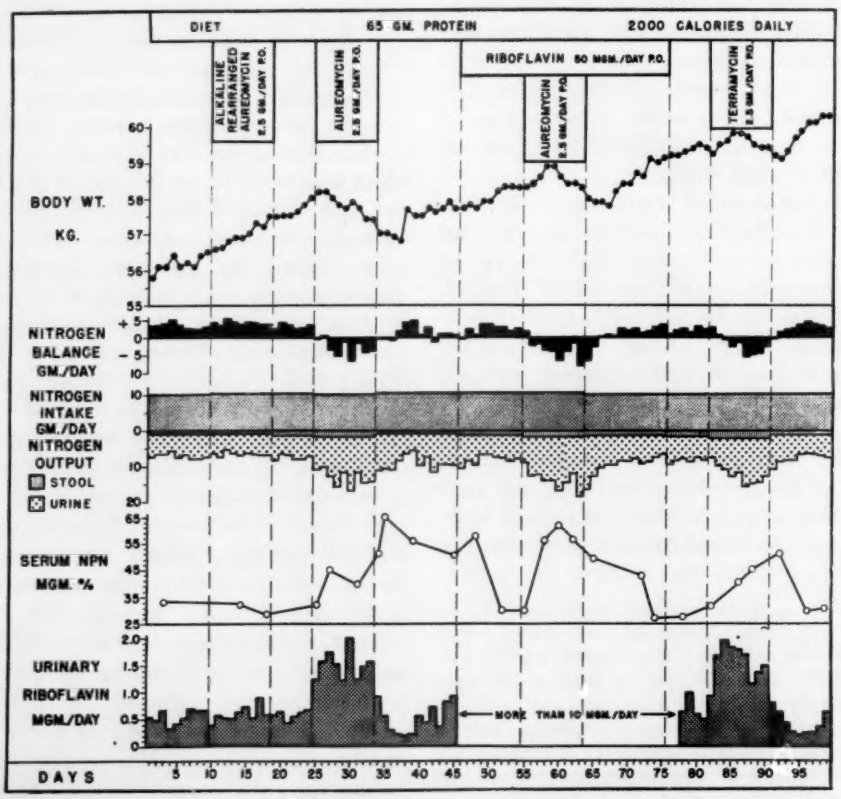

Fig. 10.—Observations on body weight, nitrogen balance, serum nonprotein nitrogen, and urinary excretion of riboflavin in Patient 5.

duce a consistently positive nitrogen balance of 3 to 5 gm. daily and a steady gain in weight. The alkaline-rearranged chlortetracycline, from which more than 99% of the antibacterial activity had been removed by mild alkaline treatment, did not affect the body weight, the nitrogen balance, or the urinary riboflavin excretion. During each of two periods when the patient received 2.5 gm. of active chlortetracycline and during a third period when he received similar amounts of oxytetracycline, there was a marked increase in urinary excretion of nitrogen, enough to account for a negative nitrogen balance. Associated with these changes in nitrogen there were also losses of body weight, beginning on the third or fourth day of the administration of the antibiotics and continuing for a similar length of time into the control periods after the antibiotics were discontinued. Administration of these antibiotics without added riboflavin was also accompanied by an increase in urinary excretion of riboflavin, which, as in the previous experiments, was about two and one-half times as great as that observed during control periods.

In this patient similar doses of oxytetracycline and chlortetracycline produced comparable losses of body weight and increases in urinary excretion of nitrogen and riboflavin, with negative nitrogen balances of the same magnitude.

During one of the periods when 2.5 gm. daily of active chlortetracycline was given and in the periods immediately preceding and following it, the patient was also given 50 mg. of riboflavin daily by mouth. The riboflavin did not alter the positive nitrogen balance or gain in weight when given alone and did not prevent the increase in urinary excretion of nitrogen and the negative nitrogen balance resulting from the chlortetracycline administration.

The levels of nonprotein nitrogen in the serum rose considerably during each of the three periods when 2.5 gm. daily of active chlortetracycline or oxytetracycline was given and returned to control levels after these antibiotics were stopped, although the elevated level persisted for several days after the first course of active chlortetracycline. The rise in nonprotein nitrogen in the serum was not quite so great or so persistent when the patient received oxytetracycline as when he was taking chlortetracycline (Fig. 10).

Nutritional Effect of a Small Dose of Chlortetracycline.—Not shown in Figure 10 are the observations made during the administration of 150 mg. of active chlortetracycline daily over a period of 15 days (from the 110th to the 124th days of the study) and during the succeeding control period of 9 days of the diet alone. Throughout these periods the body weight rose steadily from 60.6 to 62.2 kg. while the patient received these small doses of chlortetracycline and further to 62.7 kg. in the next nine days. Urinary excretion of nitrogen, the positive nitrogen balance, and the level of nonprotein nitrogen in the serum were unaffected by the chlortetracycline. Urinary excretion of riboflavin was likewise unaffected; the average daily excretion of this vitamin was 0.47 mg. while he was receiving the small doses of chlortetracycline, and it was 0.47 and 0.78 mg. during the nine-day periods immediately preceding and following the treatment, respectively.

Other Vitamins and Amino Acids.—Excretion of nicotinic acid in the urine and of riboflavin, nicotinic acid, pyridoxine, and folic acid in the feces were also measured during several periods. These data, as well as those for nitrogen balance and urinary riboflavin excretion, are summarized in the lower part of Table 7. Fecal excretion of riboflavin was not significantly altered during the various antibiotic regimens as compared with control periods. No definite trends were apparent with respect to the excretion of the other vitamins in relation to the administration of the antibiotics.

Daily urinary excretion of tryptophane and histidine was studied during the periods of administration of inactive and active chlortetracycline and during the control pe-

TABLE 7.—*Summary of Data on Nitrogen Balance and Excretion of Vitamins in Patients 1 to 5*

Patient	Days of Study	Antibiotics, Daily Dose	Nitrogen, Average Gm./Day					Vitamins, Average Daily Excretion							
			Intake		Output		Balance	Riboflavin, Mg.		Nicotinic Acid, Mg.		Pyridoxine, μg.		Folic Acid, μg.	
			Diet	Other	Urine	Feces		Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces
1	1–6		12.2	0.0	5.7	1.0	+5.5	0.42	0.80	1.01	2.23	51.2	178	9.2	168
(Fig. 1)	7–15	Chlortetracycline, 3.0 gm.	12.2	0.2	7.1	1.2	+4.1	1.07	1.01	0.86	2.90	47.6	101	7.5	129
	16–27		12.2	0.0	6.9	0.9	+4.4	0.45	0.83	0.81	2.67	39.1	118	5.6	106
2	1–9		12.2	0.0	6.6	0.9	+4.7	0.57	0.91	0.95	1.09	35.5	103	2.6	112
(Fig. 3)	10–18	Chlortetracycline, 2.5 gm.	11.6	0.1	8.5	0.8	+2.4	1.34	0.50	0.81	1.11	34.0	74	3.6	63
	19–27		12.2	0.0	7.0	0.7	+4.5	0.37	0.52	0.69	1.80	30.2	122	2.7	79
3	1–9		11.5	0.0	7.1	0.5	+3.9	0.6	0.46	0.89	1.26	49.5	153	4.	98
(Fig. 4)	10–18	Chlortetracycline, 3.0 gm.*	11.5	0.2	10.9	0.8	0.0	1.57	0.62	0.84	1.01	16 mg.	890	3.7	45
	19–27		11.5	0.0	6.8	0.4	+4.3	0.31	0.31	0.59	0.84	226	174	3.4	43
	1–9		12.9	0.0	6.7	1.2	+5.0	0.52	1.56	0.52	1.80	40.5	180	2.4	178
	10–18	Bacitracin, 100,000 units, + polymyxin, 500 mg.	12.9	0.2	9.2	1.9	+4.0	0.63	1.04	0.53	1.54	49.4	278	3.5	168
2	19–26	Bacitracin, 100,000 units, + polymyxin B, 500 mg., + chlortetracycline, 2.5 gm.	12.9	0.3	12.2	1.8	−0.8	1.47	0.72	0.69	0.78	48.2	65	5.8	113
(2d study) (Fig. 6)	27–35	Bacitracin, 100,000 units, + polymyxin, 500 mg.	12.9	0.3	7.8	1.5	+3.8	0.43	0.52	0.54	1.41	37.9	156	3.4	158
	36–44		12.9	0.0	6.7	0.8	+5.4	0.74	0.84	0.63	1.60	42.3	109	3.4	182
	1–6		12.5	0.0	8.7	1.2	+2.6	0.83	0.95	1.57	2.83	31.4	260		214
	7–15	Bacitracin, 150,000 units, + polymyxin, 500 mg.	12.8	0.3	8.8	3.2	+1.1	0.83	1.37	1.28	3.15	33.5	368		305
4	16–24		12.8	0.0	8.7	1.1	+3.0	0.69	1.08	1.07	2.84	37.5	200		112
(Fig. 9)	25–33	Oxytetracycline, 2.5 gm.	12.7	0.1	11.9	1.6	−0.7	0.71	1.56	1.13	3.20	35.5	111		373
	34–42		12.8	0.0	8.3	1.9	+2.6	0.70	0.96	0.86	3.85	29.2	133		229
	43–51	Chlortetracycline, 2.5 gm.	12.8	0.1	12.1	3.4	−2.6	1.16	2.40	0.99	3.90	37.5	550		290
	1–9		10.3	0.0	6.1	0.8	+3.4	0.56	0.65	0.55	1.11	30.4	182		153
	10–18	Alkaline-rearranged chlortetracycline, 2.5 gm.	10.4	0.1	5.5	0.9	+4.1	0.60		0.50		30.5			
	19–24		10.4	0.0	6.0	1.4	+3.0	0.59	1.07	0.54	2.31	29.5	215		104
	25–33	Chlortetracycline, 2.5 gm.	10.3	0.1	11.4	1.7	−2.7	1.53	1.07	0.70	3.11	37.5	187		98
	34–45		10.5	0.0	8.3	0.9	+1.3	0.57	0.67	0.63	1.67	27.6	133		100
	46–54	Riboflavin, 50 mg.	10.5	0.0	7.0	1.2	+2.3								
5	55–63	Riboflavin, 50 mg., + chlortetracycline, 2.5 gm.	10.5	0.1	11.9	1.7	−3.0								
(Fig. 10)	64–75	Riboflavin, 50 mg.	10.4	0.0	8.4	1.2	+0.8								
	76–81		10.4	0.0	7.0	1.4	+2.0	0.69	1.04	0.73	1.14		203		230
	82–91	Oxytetracycline, 2.5 gm.	10.4	0.1	10.3	2.0	−1.8	1.52	1.36	0.70	3.02		136		144
	92–109		10.5	0.0	5.8	1.1	+3.6	0.47	1.00	0.55	2.12		106		180
	110–124	Chlortetracycline, 150 mg.	10.5	0.0	6.5	1.1	+2.9	0.46	1.14	0.61	2.16		120		138
	125–133		10.5	0.0	7.8	1.1	+1.6								

* Contained 200 mg. of pyridoxine hydrochloride.

riods immediately preceding and following them. The results are summarized in Table 8. The data indicate that the oral administration of 2.5 gm. of active chlortetracycline daily was associated with an increase in the urinary excretion of both of these amino acids to more than twice the average daily amounts excreted in control periods. The inactive chlortetracycline had no such effect.

Other Clinical Laboratory Findings.—The following additional determinations were made on repeated occasions during each of the control and treatment periods: hemoglobin, hematocrit, red blood cell count, total and differential white blood cell count, and the usual urinalysis, including pH and urobilinogen concentration. None of the data revealed any changes related to any of the antibiotic regimens, except for an increase from 2% to 11% in the eosinophils that occurred only during the period when the alkaline-rearranged chlortetracycline was given.

Character of the Stools.—All of the stools were formed, but they were softer and bulkier during all of the periods when active antibiotics were being given. In the first two control periods and the intervening one during which the inactive chlortetracycline was given, the average daily weight of the fecal output was 67, 71, and 65 gm., successively; it averaged 170 gm. during the first period of active chlortetracycline administration, from 129 to 150 gm. daily during the other periods when active antibiotics were given, and 100 gm. or less during the other control periods.

TABLE 8.—*Effect of Oral Administration of Active and Inactive Chlortetracycline on the Urinary Excretion of Tryptophane and Histidine in Patient 5*

Period	Days of Observation	Antibiotic *	Average Daily Urinary Excretion, Mg. †	
			Tryptophane	Histidine
I	1–9	None	6.8	27
II	10–18	CTC (AR)	7.3	27
III	19–24	None	6.8	25
IV	25–33	CTC	16.7	55
V	34–45	None	7.6	26

* CTC indicates chlortetracycline, 2.5 gm. daily; AR, alkaline-rearranged (inactive).
† Based on last 6 days of each period.

Bacteriologic Studies of the Feces.—Extensive studies were made of the aerobic and anaerobic flora on several specimens of feces during each period of the study. The results will be summarized briefly; some of the more striking quantitative findings are shown in Figure 11.

Gram Stains.—In Gram-stained smears of the fresh feces, no striking changes were noted in relation to therapy. Gram-positive rods predominated throughout except during the last days of oxytetracycline therapy, when Gram-positive cocci predominated. Two or three yeast forms per 100 organisms were counted intermittently throughout the study, but the greatest number of them were seen during the period when the patient was receiving chlortetracycline and riboflavin.

Total Number of Organisms.—The total number of organisms, as judged from pour-plate counts, ranged in the different specimens from 10^7 to nearly 10^{10} per gram of feces and did not in general appear to be related to the treatment being used at the time.

Coliform Organisms.—The Gram-negative bacillary forms grown during this study were predominantly E. coli and A. aerogenes, the latter being the most numerous. The total number of coliforms did not change markedly during the ingestion of the alkaline-rearranged chlortetracycline and during the first period, when 2.5 gm. of active chlortetracycline was given alone. However, they could not be demonstrated at the end of the period when chlortetracycline and riboflavin were given and briefly thereafter but then recurred. They were again essentially eliminated soon after oxytetracycline was started and were not observed until six days after that treatment was stopped; after that they persisted throughout the remainder of the study and were not affected by the small doses of active chlortetracycline.

Gram-Positive Organisms.—Enterococci were present during the first three periods, including the nine days of administration

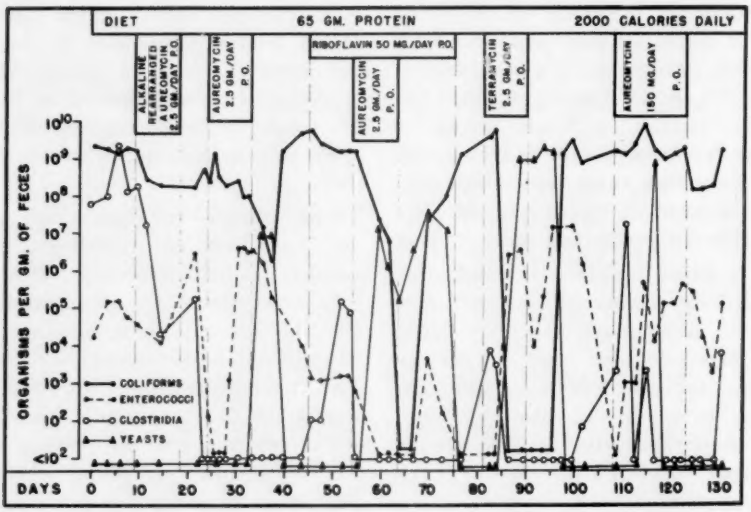

Fig. 11.—Effect of various antibiotic regimens on certain elements of the fecal flora in Patient 5.

of the inactive chlortetracycline. They could not be demonstrated during and immediately after the subsequent administration of 2.5 gm. daily of active chlortetracycline or oxytetracycline and were temporarily reduced during the first few days of treatment with 150 gm. of chlortetracycline daily. Clostridia were most consistently affected by the administration of the active tetracycline antibiotics, including the small doses of chlortetracycline. Organisms of the Bacillus subtilis group were grown in small numbers, averaging 10^2 to 10^3 per gram of feces, during control periods but were not identified in any of the stools obtained during treatment with the active antibiotics.

The most striking finding, as shown in Figure 11, was the inverse relationship between the growth of yeasts and of the coliforms. Each marked drop in the numbers of the latter was associated with the cultivation of moderate or large numbers of yeast (mostly Candida), which were not grown during the periods when the coliforms were abundant.

Antibiotics in Feces.—From 1.5% to 12% of the daily intake of 2.5 gm. daily of chlortetracycline was accounted for during that therapy as active antibiotic in the feces; some of the antibiotic was still demonstrable in the stools six days but not eight days after the therapy was stopped. During the administration of 150 mg. of chlortetracycline daily, 0.4% to 0.6% of the daily intake was recovered in the stools, and none was detectable after that treatment ended.||

Summary.—In this patient it was shown that chlortetracycline deprived of its antibacterial activity by alkaline treatment failed to influence the body weight and urinary excretion of nitrogen and riboflavin. Active chlortetracycline or oxytetracycline in oral doses of 2.5 gm. daily produced a loss of weight, increased urinary excretion of nitrogen, change from positive to negative nitrogen balance, rise in nonprotein nitrogen of the serum, and a 2-and-one-half-fold increase in urinary excretion of riboflavin without consistently affecting the excretion of pyridoxine, nicotinic acid, or folic acid. A daily oral dose of 150 mg. of active chlortetracycline failed to produce these effects. Administration of riboflavin alone had no effect on the nitrogen balance and

|| The method used is subject to large quantitative errors.

did not prevent the change from a positive to a negative nitrogen balance during treatment with 2.5 gm. daily of active chlortetracycline. The oral administration of 2.5 gm. of chlortetracycline also resulted in a two-fold or greater excretion of tryptophane and histidine, and this did not occur when the inactive chlortetracycline was given.

Bacteriological studies of the feces showed no marked or persistent changes in the total bacterial counts, but the administration of active chlortetracycline or oxytetracycline was associated with marked reductions in the numbers of coliforms (E. coli and A. aerogenes), enterococci, and clostridia, along with the demonstration of varying numbers of yeasts (Candida). All of these returned to their pretreatment status during or soon after the antibiotics were discontinued. The most marked and most prolonged effects were on the clostridia. The inactive chlortetracycline failed to produce any of these effects, whereas the daily oral doses of 150 mg. of active chlortetracycline was associated with a reduction in the number of clostridia to below demonstrable levels without significantly affecting the numbers of enterococci, coliforms, or yeasts.

PATIENT 6.—The patient, an undernourished man weighing 54.6 kg., was studied over a period of two months, during which he was maintained on a constant diet that provided about 80 gm. of protein and 3100±100 Cal. daily. During this time, observations were made of the effect on body weight, urinary nitrogen excretion, and fecal bacterial flora as affected by three dosage regimens of chlortetracycline, namely, (1) a daily total of 20 mg. orally for 15 days, (2) 1 gm. intravenously each day for 6 days, and (3) a total of 1 gm. orally each day for 6 days. These periods were each preceded and followed by a control period when the patient received the same diet without antibiotics. Urinary excretion of riboflavin was also measured during five of the seven periods.

Body Weight and Urinary Excretion of Nitrogen and Riboflavin (Table 9).—There was an apparently irregular increase in weight throughout the 63 days of this study. However, the small dose of antibiotic did not interfere with the gain in weight, but the periods of treatment with the 1.0 gm. daily doses were brief and the effects on weight overlapped into the succeeding period, there being a decline in weight at the end of these periods and during the next four days. During the 15 days when the patient was receiving 20 mg. of chlortetracycline daily by mouth the gain in weight was at about the same rate as in the control periods immediately preceding and following; the daily excretion of nitrogen in the urine, however, was increased somewhat over that of the control period immediately preceding and following. The gain in weight was not interrupted during the six days of administration of 1 gm. of the antibiotic intravenously or later when the same dose was given orally for six days; however, the urinary excretion of nitrogen was increased to a greater extent during each of these periods as compared with the time

TABLE 9.—*Body Weight and Urinary Excretion of Nitrogen and Riboflavin in Patient 6*

Period	Days of Observation	Antibiotic *	Average Weight Gain, Gm./Day	Average Daily Intake		Average Daily Output in Urine		Average Weight of Feces, Gm./Day
				Cal.	Nitrogen, Gm.	Nitrogen, Gm.	Riboflavin, Mg.	
I	1-9	None	156	3,220	12.7	6.0	--	223
II	10-24	CTC, 20 mg./day	147	3,115	12.7	7.7	--	250
III	25-36	None	83	3,110	12.7	6.4	0.41	215
IV	37-42	CTC, 1 gm./day intravenously	89	3,060	12.8	9.5	0.80	293
V	43-51	None	67	3,080	12.7	6.8	0.36	264
VI	52-57	CTC, 1 gm./day orally	183	3,050	12.8	8.2	0.65	344
VII	58-63	None	12	3,020	12.7	6.6	0.35	290

* CTC indicates chlortetracycline.

TABLE 10.—*Bacteriologic Observations on Feces of Patient 6**

Period	Day of Study	Stained Smear: Gram + Cocci, %	Stained Smear: Bacilli, % Gram +	Stained Smear: Bacilli, % Gram —	Total Aerobes, ×10⁷	Coliforms, ×10⁶	Enterococci, ×10⁶	S. Pyogenes † Aureus, ×10⁵	S. Pyogenes † Albus, ×10⁵	Bacillus Subtilis, ×10⁵	Total Anaerobes, ×10⁷ ‡	Clostridia, ×10⁷	Yeasts, ×10⁷ §	CTC, μg./Gm.
I	1	..	..	..	1.6	6.0	0.05	..	..	1.3	..	0.9	..	
Diet alone	3	52	27	67	14.0	7.7	0.15	0	2.5	0.1	15	0.04	0.75	
	5	11	38	51	2.5	6.1	0.08	0	2.0	0	16	0.2	0.75	
	9	6	20	74	1.7	1.4	0	0	2.5	0	17	1.6	0	
	10	9	52	39	0.13	0.2	0	0	3	0	130	0.11	0.75	0 ‖
II	11	4	29	62	0.54	1.2	0	0	5	0	90	0.42	6.5	0
CTC,	13	6	7	86	0.85	4.9	1.1	..	..	10	..	0.07	..	..
20 mg./day	15	1	33	65	2.3	15	6.5	25	6	0	140	0.16	26	..
orally	17	3	50	47	2.1	17	4.0	..	..	0	..	0.002	..	0
	19	2	33	65	0.97	2.4	7.3	5	7.5	0	50	0.00002	42	..
	21	11	29	60	8.8	7.6	8.0	25	25	0	23	0.0003	180	..
	23	13	19	68	0.72	2.9	4.3	23	28	44	2	0	29	..
	25	21	25	54	1.0	6.4	3.7	0	18	17	8	0	7.5	
	26	24	32	44	6.0	5.3	8.3	..	..	6	..	4.6	..	
III	28	33	25	43	0.52	1.4	1.6	0	23	0	7	0.22	1.5	
Diet alone	31	15	36	51	0.28	0.5	2.2	0	7.5	17	22	0.01	2.0	
	33	16	26	58	0.74	4.5	2.9	0	0.3	3	4	0.00	4.0	
	36	12	7	81	3.3	20	0.5	0	4	21	7	1.3	2.5	
	37	13	27	60	0.3	2.0	0	0	5.5	6	3	0.03	2.5	0
IV	38	21	32	47	0.16	1.6	0	0	8	0	10	0	11	64
CTC,	39	9	30	61	0.10	0.6	0	0	11	0	3	0	19	64
1 gm./day	40	13	34	53	0.10	0.9	0.12	0	9	1	14	0	400	32
intravenously	41	19	40	41	0.42	0.2	4.0	0	25	0	4	0	85	16
	42	22	15	63	1.5	0.6	15	0	65	4	10	0	35	8
	43	29	28	43	8.6	1.2	84	0	7	8	10	0	4.8	0
V	45	63	20	17	1.8	4.2	14	0	1	2	25	0	28	
Diet alone	47	54	35	11	1.9	3.7	15	0	1.5	0	29	0.006	18	
	50	57	27	16	3.0	3.3	27	0	1	3	5	0.0001	9.5	
VI	52	60	31	9	1.5	0.8	11	0	4.8	0	1	0	29	250
CTC,	53	74	19	7	19	1.1	192	13	46	0	33	0	69	500
1 gm./day	54	59	15	24	46	0.3	456	1.5	8.5	0	12	0	33	125
orally	56	27	29	44	176	0.04	1720	10	25	0	210	0.00005	43	250
	57	14	34	52	69	0.7	690	13	10	0	160	0	38	250
VII	59	61	21	18	10	2.4	103	1	16	0	70	0.0008	85	0
Diet alone	61	65	16	19	2.1	2.8	18	0	1.5	21	35	0.00006	67	
	63	53	18	29	2.4	2.3	20	..	..	0	..	0.0002	..	

* All counts are per gram of feces; CTC indicates chlortetracycline; .. = not done
† On Staphylococcus medium (Difco), done by Dr. Loh.
‡ On Brewer's anaerobic agar, done by Dr. Loh.
§ On grape-juice agar, done by Dr. Loh.
‖ 0 indicates <8μg./gm.

when the small oral doses were ingested, and it was greatest during the intravenous administration of the antibiotic. Urinary excretion of riboflavin was definitely increased during the regimens of 1 gm. of chlortetracycline daily; twice as much was excreted during the intravenous period and about 85% more when the dose was given orally, as compared with the three control periods.

The nonprotein nitrogen level of the serum fluctuated somewhat (24±3 mg. per 100 ml.) throughout this study but without relationship to any of the antibiotic regimens.

Character of the Stools.—The bulk of the fecal output increased during each of the periods of chlortetracycline administration; this increase was only slight during the ingestion of the small oral doses and was greatest when doses of 1 gm. were given orally (Table 9). The increased weight of the stools continued during the six days after these doses were stopped.

Bacteriological Observations on the Feces (Table 10).¶—The distribution of different morphological forms of bacteria in the feces, estimated from Gram-stained smears, showed no clear correlation with the various treatment regimens. The proportion of Gram-negative bacilli was lower and that of Gram-positive cocci greater during the oral administration of the 1 gm. doses of chlortetracycline, but this difference was first noted during the preceding control period.

The total number of aerobic bacteria decreased slightly at the beginning of each of the three antibiotic treatment periods but soon returned to pretreatment levels; during the 1 gm. oral doses there was a further one-hundred-fold increase. The total number of anaerobes likewise increased during the latter period but otherwise showed no important changes.

Coliform organisms (only E. coli was identified throughout this study) decreased somewhat in number during the 1 gm. doses, both intravenous and oral. Enterococci could not be demonstrated during the first half of each of the first two treatment periods but reappeared and increased during the latter half of these periods. No definite decline in the number of these organisms was made out during the 1 gm. oral doses; instead their number increased about three logarithms during this period. Micrococcus pyogenes var. albus was cultured in small numbers throughout all periods, but Micrococcus pyogenes var. aureus was identified also in small numbers only during the oral administration of chlortetracycline; the latter were all hemolytic and coagulase-negative.

Aerobic spore-forming Gram-positive bacilli (Bacillus subtilis) were grown irregularly and in small numbers in each period except during oral therapy with the daily doses of 1 gm. Clostridia were decreased markedly during the small oral doses and could not be grown in the latter days of that treatment; they were essentially eliminated from all cultures of feces obtained while the 1 gm. doses were given, either intravenously or orally. Yeasts were grown by Dr. Loh throughout the study; they appeared to increase about one-hundred-fold during the first two treatment periods but not while the doses of 1 gm. were given orally.

Sensitivity of Fecal Organisms to Chlortetracycline.—Strains of enterococci isolated during each of the control periods were inhibited by 12.5μg. or 25μg. of chlortetracycline per milliliter, whereas those isolated during each of the three periods this antibiotic was administered required 400μg. per milliliter to produce complete inhibition. All of the strains of Micrococcus pyogenes var. aureus, including those isolated before the first treatment period, required 400μg. of chlortetracycline per milliliter for inhibition. Strains of E coli isolated during both control and treatment periods were inhibited by 6.25μg. per milliliter except one strain, isolated when 1 gm. was being given orally, which required 50μg. per milliliter. All

¶ Dr. Loh provided some of the data on this patient.

strains of Bacillus subtilis were inhibited by 1.6μg. to 6.25μg. per milliliter, and the strains of clostridia tested were inhibited by 12.5μg. per milliliter.

Chlortetracycline in Feces.—The antibiotic could not be demonstrated in the feces during the oral treatment with doses of 20 mg. daily. During intravenous therapy, up to 64μg. per gram and up to 4% of the daily dose were found in the stools. During oral doses of 1 gm. daily, concentration of 125μg. to 500μg. per gram were found, and from 2.5% to 12.5% of the daily dose could be accounted for in the feces.

Summary.—In this patient, the administration of oral doses of 20 mg. of chlortetracycline daily appeared to increase nitrogen excretion slightly and also affected the bacteriologic findings in the stools to an appreciable degree. The intravenous dose of 1 gm. of chlortetracycline was associated with a greater increase in urinary excretion of nitrogen and riboflavin than was the same dose given orally. None of these three doses affected the increase in weight which continued throughout the study while the patient was receiving a diet that provided about 3000 Cal. and 80 gm. of protein. Some of the changes in the fecal flora observed during the intravenous and oral administration of 1 gm. of chlortetracycline were very similar, whereas others were quantitatively different.

PATIENT 7.—This final brief study was designed primarily to reinvestigate the effect of an oral dose of 20 mg. of chlortetracycline daily on the body weight, excretion of nitrogen in the urine, and the fecal flora. The patient weighed 82.3 kg. at the start of the study. He was maintained on a constant diet that provided 76 gm. of protein and 2750 Cal. daily. The chlortetracycline was given for 15 days, preceded and followed by control periods of 11 and 7 days, respectively, on the same diet without the antibiotic. The findings are shown in Tables 11 and 12.

Body Weight and Urinary Nitrogen (Table 11).—There was a continuous gain in weight throughout the study, and this was not reversed during the chlortetracycline period. Urinary excretion of nitrogen also was not significantly affected. The average daily fecal output, however, was increased in weight when chlortetracycline was given.

Bacteriologic Findings in the Feces (Table 12).—Gram-negative bacilli predominated (82% to 96%) in the direct smear of all specimens of stools that were examined. Coliform organisms, of which only E. coli could be identified, persisted, with marked changes in number throughout all three periods. Enterococci appeared only after the fourth day on chlortetracycline and was present in moderate numbers until the last day, after which they again could not be demonstrated. Staphylococci were not cultured from any of the stools. Bacillus subtilis was grown in small numbers once immediately before and twice during the antibiotic therapy. Clostridia appeared first to be increased and then decreased during treatment and could not be cultivated in the last two specimens examined during chlortetracycline administration, but they reappeared after the antibiotic was stopped.

Summary.—The oral dose of 20 mg. of chlortetracycline daily failed to have any significant effect on the gain in weight or urinary excretion of nitrogen, although it did produce appreciable effects on the fecal bacterial flora and was accompanied by an apparent increase in the bulk of the stool.

TABLE 11.—*Body Weight and Intake and Urinary Output of Nitrogen in Patient 7* *

Period	Antibiotic	Days of Observation	Nitrogen Intake, Gm./Day	Nitrogen in Urine, Gm./Day	Feces, Gm./Day	Gain in Weight, Gm./Day
I	None	1–11	12.2	5.4	94	82
II	Chlortetracycline 20 mg./day	12–26	12.2	6.2	125	61
III	None	27–33	12.2	6.2	75	62

* Diet: 76 gm. of protein, 2,750 Cal. daily. Weight at start of study: 82.3 kg.

TABLE 12.—*Relevant Bacteriological Observations in Feces of Patient 7* *

Period	Day of Observation	Organisms/Gm. of Feces, No. Coliforms, $\times 10^8$	Enterococci, $\times 10^6$	B. Subtilis, $\times 10^2$	Clostridia, $\times 10^6$	Yeasts
	1	20	0	0	0.2	0
I	3	7	0	0	2	0
Control	7	20	0	0	--	0
	11	7	0	3	2	0
	12	55	0	0	0.2	0
	14	200	0	17	780	0
II	15	2	0	0	200	0
CTC,	16	4	5	0	23	0
20 mg./day	18	50	3	0	4	0
orally	21	100	4	0	0.06	0
	23	40	3	0	0	0
	25	40	0	9	0	10^4
III	29	5	0	0	0.02	0
Control	32	18	0	0	85	0

* CTC indicates chlortetracycline.

Comment

Chlortetracycline administered orally to undernourished men resulted in losses of body weight, increases in the excretion of nitrogen in the urine with a resultant negative or reduced positive nitrogen balance, and increased excretions of riboflavin in the urine. Results with oxytetracycline were qualitatively similar to those with chlortetracycline. Similar findings have been confirmed by other investigators for these two antibiotics [16,17] and have, in addition, been noted for tetracycline.[18,19]

Whether the metabolic effects of these tetracycline compounds are related to their antibacterial properties or result from a direct effect of these agents upon the tissues of the subjects studied which is independent of antibacterial activity is difficult to resolve. In reference to the former possibility, several studies were done to evaluate the role of the gastrointestinal flora in relation to the changes in nitrogen and riboflavin excretion observed. Although bacitracin and polymyxin given orally produced the most profound changes in the fecal flora, their administration was not accompanied by metabolic alterations similar to those observed with the tetracycline compounds. In one patient administration of bacitracin plus polymyxin produced marked changes in the fecal flora but no metabolic alterations. When chlortetracycline was given in addition to the other antibiotics there was no further discernible change in fecal flora, but the expected increases in urinary excretions of nitrogen and riboflavin occurred. Following cessation of chlortetracycline, reversal of the alterations in metabolism occurred in spite of the continued administration of bacitracin plus polymyxin, but the return of fecal flora toward the control pattern did not occur until all antibiotics were withdrawn.

Several additional findings illustrate the lack of correlation of qualitative or quantitative change in fecal flora to the metabolic changes noted with tetracycline compounds. The fecal flora changed in all patients given active antibiotics, but the alterations differed considerably in each patient. Bacitracin plus polymyxin given orally produced the most striking reduction in bacterial flora, but did not produce metabolic changes. Chlortetracycline given orally in doses of 150 mg. daily for 15 days altered the bacterial flora, but significant metabolic alterations did not occur with these smaller doses. The studies reported here failed to establish a relationship between the metabolic effects of tetracycline compounds and their effect on the flora of the intestine. However, because of the difficulties encountered in the techniques and interpretation of quantitative bacteriology of the feces, a definitive conclusion may not be justified. The bacteriologic data presented, however, would not support the hypothesis that the metabolic effects of tetracycline compounds

are mediated through their effect on the gastrointestinal flora.

The administration of antibiotics might influence the fecal excretion of vitamins by several mechanisms. Alterations in the gastrointestinal bacterial flora resulting from antibiotics may account for changes in the fecal excretion of vitamins, since bacteria synthesize and utilize such nutrients. Antibiotics might also alter the digestion or absorption, and thereby the availability, of nutrients ingested with food. Changes were noted in the present study in both the gastrointestinal bacterial flora and the character of the stools in relation to antibiotic administration. Stools collected during antibiotic therapy were usually bulkier and heavier and contained increased amounts of undigested food and fibrous material; thus the antibiotics employed may have interfered with digestive processes of the intestinal tract in some way not related to action of bacteria. However, these changes do suggest that digestion is normally, in part at least, a function of the gastrointestinal bacterial flora. The data are not adequate enough to permit definitive clarification of these possibilities.

These considerations and the technical difficulties encountered in assaying stool specimens quantitatively for their content of vitamins limit the interpretation of data. However, the following points are of interest. The fecal excretions of some vitamins were significantly altered without an accompanying alteration in the excretion of the respective vitamins in the urine. Fecal excretions of the vitamins studied were not reduced to negligible quantities and, to the contrary, were sometimes increased by antibiotic therapy. The fecal excretions of the vitamins were not altered in any consistent direction in relation to the administration of the antibiotics. In any event, the increased urinary excretion of riboflavin resulting from tetracycline administration cannot be explained on the basis of an increased absorption of ingested riboflavin or an enhanced bacterial production of this nutrient, since the fecal excretion of this substance was variably altered in the patients studied.

The increases in urinary nitrogen that occurred in conjunction with the administration of tetracycline drugs are likewise difficult to explain by an effect of the antibiotics on the digestion or absorption of food nitrogen, since the fecal excretion of nitrogen was either not significantly reduced or was sometimes even increased during periods of administration of these antibiotics. In fact, the largest fecal nitrogen excretions occurred with the nonabsorbable antibiotics; only part of this could be accounted for by nitrogen from the polypeptide antibiotics. Moreover, the unabsorbed antibiotics had no significant effect on excretion of nitrogen in the urine. If anything, one would expect increases in stool nitrogen to be accompanied by no change or by decrease in the excretion of nitrogen in the urine. The increases in concentration of nonprotein nitrogen in serum that occurred during administration of chlortetracycline and in face of adequate outputs of urine suggest that the observed increases in excretion of urinary nitrogen are due to a prerenal effect of the drug, rather than some primary influence it might have on the renal clearance of nitrogen. This is further supported by the losses of body weight that were associated with the increased nitrogen losses.

The urinary excretions of tryptophane, histidine, and threonine were increased in relation to chlortetracycline administration. Although the mechanism responsible for these increases and their significance are not apparent, the magnitude of increases in the excretion of these essential amino acids was not great enough to be of nutritional significance.

Since there is a close relationship between riboflavin and the metabolism of tissue protein,[20] it is possible that the increases in urinary riboflavin excretion noted with the tetracycline compounds are related to the increases in urinary excretion of nitrogen.

However, in most cases the urinary riboflavin excretion preceded the increases in excretion of urinary nitrogen and in the concentrations of serum nonprotein nitrogen that occurred with chlortetracycline administration. The magnitude of the increased urinary excretions of riboflavin was also in excess of that noted for nitrogen.

Some of the studies were also designed to test the effect of riboflavin supplements on the metabolic changes resulting from chlortetracycline. The oral administration of large amounts of riboflavin before and during ingestion of the antibiotic did not prevent the metabolic effects of the latter. However, in one patient (Patient 5, Fig. 10) the first course of active chlortetracycline was accompanied by increased excretions of urinary nitrogen and riboflavin and increases in concentrations of serum nonprotein nitrogen. These changes reverted to control levels more slowly following cessation of chlortetracycline than they did following the second course of chlortetracycline, when the oral riboflavin supplement was continued; likewise, this patient's rate of gain in body weight was less after chlortetracycline was stopped than that noted during the initial control periods; but when riboflavin was given with and after the antibiotic the usual rate of weight gain followed the discontinuance of the drug.

It might be suggested then that the increases in urinary nitrogen excretion noted with the tetracycline antibiotics are secondary to some influence these agents have upon riboflavin-dependent enzyme systems that may be necessary for the maintenance of tissue proteins or their synthesis. This implies that the tetracycline compounds may exert their metabolic effects directly on the tissues, rather than indirectly through their influence on the normal bacterial flora of man. This does not imply that the basic mechanism of action of these antibiotics in relation to the metabolic observations reported here and to their bacteriostatic action may not involve common biochemical systems. Although the precise mechanism whereby tetracycline compounds are bacteriostatic is not known, an influence of riboflavin upon the sensitivity of certain organisms to chlortetracycline has been reported.[21]

The metabolic effects of tetracycline administration noted in the present study utilizing relatively large doses of these agents are in contrast to the reports of other workers indicating a growth-enhancement effect of smaller doses of these agents in undernourished children and in various animal species.[1] Two of the undernourished adult patients were studied during administration of small doses of chlortetracycline orally to determine whether or not a "growth enhancing" effect might occur. This did not occur, since rates of body weight gain and nitrogen balance were not significantly influenced by the smaller doses. Contrariwise, attempts to produce "catabolic" responses in chickens and rats given large amounts of chlortetracycline and tetracycline have been unsuccessful.[18,22]

Finally, since losses of body weight and increases in urinary nitrogen and riboflavin excretions occur with chlortetracycline, oxytetracycline, and tetracycline, as reported here and elsewhere,[16-19] the cyclic structure of these agents rather than their attached peripheral groups appears responsible for the observed metabolic changes.

Summary

Nutritional Effects.—Chlortetracycline given to undernourished adult men in doses of 2.5 or 3.0 gm. orally daily resulted in losses of body weight, increases in the excretion of nitrogen in the urine, either no change or slight increases in fecal output of nitrogen, and negative nitrogen balances. The increased excretion of nitrogen in the urine was accompanied by increases in the concentration of nonprotein nitrogen in the serum. Similar changes resulted from comparable doses of oxytetracycline and from 1.0 gm. of chlortetracycline given either orally or parenterally (one patient),

although body weights and serum nonprotein nitrogen concentrations were not detectable altered by the latter. A dose of 2.5 gm. of alkaline-rearranged chlortetracycline that is microbiologically inactive, small oral doses of active chlortetracycline (20 or 150 mg. daily), and bacteriologically effective doses of bacitracin plus polymyxin given orally did not produce such metabolic changes. Orally administered bacitracin and polymyxin influenced nitrogen balance slightly by increasing the output of nitrogen in the stools, but had no significant effect upon urinary excretion of nitrogen.

Marked increases in the excretion of riboflavin in the urine consistently occurred following the administration of chlortetracycline (1.0 to 3.0 gm. daily), and, in one of two patients, following oxytetracycline (2.5 gm. daily). The smaller doses of chlortetracycline were without this effect. The increased excretions of riboflavin usually preceded the increases in the excretion of nitrogen in the urine. The urinary excretion of pyridoxine and nicotinic acid (niacin) were not altered by the administration of antibiotics. There was an increased excretion of folic acid in the urine associated with chlortetracycline in one of four studies in which the measurement was made. Urinary excretion of N′-methylnicotinamide was determined in two patients who were given chlortetracycline; suggestive increases in the excretion of this metabolite of niacin were noted in both of them.

The fecal excretion of vitamins was not altered in any consistent direction in relation to the administration of antibiotics. Fecal excretion of riboflavin either decreased slightly, remained unchanged, or increased slightly; pyridoxine excretion remained unchanged or decreased, and folic acid excretion remained unchanged or decreased in most patients but increased in one. Fecal excretion of nicotinic acid was usually not significantly altered, except for a decrease noted in one patient. The changes in the fecal excretions of these vitamins had no apparent relationship to their excretion in the urine.

Urinary excretion of the 10 "essential" amino acids was determined for three patients and of tryptophane and histidine in an additional patient before, during, and after chlortetracycline. Increased excretions of tryptophane, histidine, and threonine were associated with the administration of this antibiotic, but otherwise no consistent or significant changes were noted in the excretion of amino acids.

In seven of the eight patients, the bulk and weight of the stools increased during administration of the antibiotics, and this increase usually persisted into the immediate post-treatment period. These stools usually had an increased amount of undigested food particles and fibrous material and had less odor than pretreatment stools.

Bacteriologic Effects.—Direct Gram-stained smears of feces usually showed very little if any consistent change in relation to antibiotic administration and frequently failed to reflect changes that were demonstrated in cultures. The average total numbers of organisms per gram of feces usually varied from 10^8 to 10^9 and showed no consistent relation to antibiotic administration.

The organisms most frequently found in the feces were as follows: coliforms (Escherichia coli and Aerobacter aerogenes), enterococci, Bacillus subtilis, and clostridia (Clostridium welchii). Those that appeared frequently during or after treatment were as follows: hemolytic Micrococcus pyogenes var. aureus (coagulase-negative), Proteus, and yeasts (Candida albicans). There was no consistent change in the number and varieties of organisms recovered during or after the different antibiotic regimens employed. Clostridia, however, were markedly decreased in number or entirely absent in five of seven patients during antibiotic therapy. Marked differences in the numbers and varieties of organisms could be obtained in the same specimens of feces, depending on the techniques used.

Considerable concentrations of antibiotics could be demonstrated in the feces during treatment, and the amounts of chlortetracycline accounted for in the feces varied with the dose and route of administration. The antibiotics were still demonstrable in the feces from one to five days after therapy was stopped. Organisms that first appeared in the stools after antibiotic therapy was started were usually markedly resistant to the antibiotic that was given.

Dr. Charles S. Davidson provided continuous interest and encouragement in this work. The microbiological studies were carried out with the technical assistance of Elaine A. Hirschberg, Leonore DeCarli, Alice N. Ballou, Ellen J. Doyle, and Virginia Olson. The bacteriological studies were done with the assistance of Clare Wilcox and Marilyn K. Broderick. The dietetic aspects of the studies were managed by Barbara Wolfe and Mrs. J. Spencer Rochfort.

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News and Comment

ANNOUNCEMENTS

Fifth International Congress of Internal Medicine.—The Fifth International Congress of Internal Medicine will be held in Philadelphia, April 23-26, 1958. Information regarding this meeting and The International Society of Internal Medicine may be obtained from Dr. Edward R. Loveland, 4200 Pine St., Philadelphia 4.

This is the first meeting of the Society to be held in this country. Another is not likely to be held here for a decade. It was arranged after an invitation was officially extended by the American College of Physicians and is intended to encourage a greater participation of the American physicians in the International Society and to give foreign members a first-hand opportunity to learn more about American developments in medical sciences. In the past six months 1900 American physicians have joined the Society, which now numbers 4000 members, from 34 nations of the world.

American Trudeau Society Meeting.—Three days of scientific sessions will mark the 53d Annual Meeting of the American Trudeau Society, medical section of the National Tuberculosis Association, Monday through Wednesday, May 19-21, 1958, in Philadelphia. On May 22, the Society will meet jointly with the National Tuberculosis Association at sessions which will bring the meetings of the two associations to a close.

In addition to papers by clinicians and investigators from universities and medical centers throughout the country, there will be panels on the tuberculin test, fungus diseases of the lung, and radiation hazards. A special talk will be given Tuesday, May 20, by Dr. Harriet Hardy, of Massachusetts General Hospital, Boston, on "Respiratory Disease in Europe, Africa, and Peru in 1957."

Sessions will be held at the Convention Hall, except for "Sandwich Seminars," which will be conducted during the lunch period Monday and Wednesday at nearby hospitals. Arrangements have also been made for a limited number of physicians to visit the Cardiac Research Unit of Philadelphia General Hospital, Blockley Division; Jefferson Hospital for a demonstration of extracorporeal devices in open heart surgery by Dr. John Gibbon; the Hahnemann Hospital where Dr. J. Stauffer Lehman will demonstrate cardiac ventriculography, and Woman's Medical College Hospital, where cases of sarcoidoses will be presented by Dr. Maurice Sones.

American Board of Nutrition.—The American Board of Nutrition will hold the next examinations for certification as a Specialist in Human Nutrition during the week of April 13-19, 1958, at Philadelphia. Candidates who wish to be considered for these examinations should forward applications to the Secretary's office not later than March 1. Application forms may be obtained from the Secretary, Otto A. Bessey, Environmental Protection Research Division, Quartermaster Research and Development Center, Natick, Mass.

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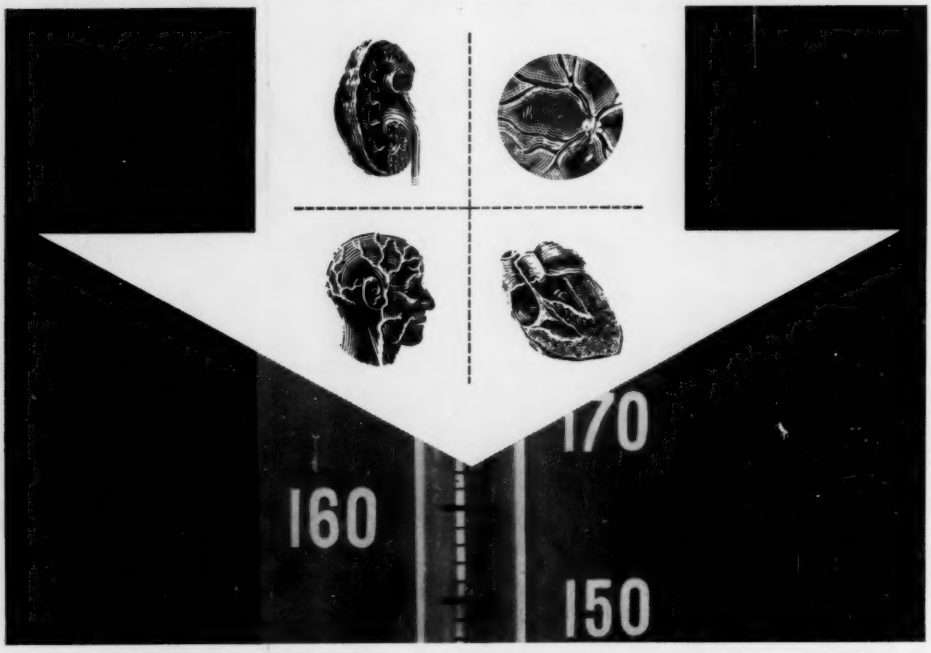

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In addition to lowering pressures in 88% of patients with diastolic readings of 140 or higher, Methium therapy has produced substantial improvements in related symptomatology (see table).[1,2] The value and safety of Methium has been demonstrated in long-term management—first to lower, then stabilize blood pressure while arresting many cardinal symptoms associated with renal, cardiac and visual functions.[1,3,4]

Methium, a potent autonomic ganglionic blocking agent, should be administered only under careful supervision.[2] However, with a thorough evaluation of the patient and subsequent adjustment of dosage to individual needs, Methium is a dependable, well tolerated and highly effective hypotensive agent.

Symptom or sign	No. Patients Complaining	Patients Improved
Headache	39	30 (79%)
Precordial pain	31	22 (71%)
Eye ground changes	53	36 (68%)
Heart failure	35	26 (72%)
Abnormal EKG strain	48	32 (67%)

Summary, by symptomatologic categories, of improvements reported by investigators using Methium therapy for severe essential and malignant hypertensive patients.

References: **1.** Moyer, J. H.; Miller, S. I. and Ford, R. V.: J.A.M.A. *152*:1121 (July 18) 1953; **2.** Kuhn, P. H.: Angiology *4*:195 (June) 1953. **3.** Ford, R. V. and Spurr, C. D.: Am. Pract. *5*:251 (April) 1954. **4.** Wolfe, J. B.; Walkow, M. D.; Nagler, J. H. and Anastasia, J.: J. Am. Geriatric Soc. *2*:365 (June) 1954.

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The authors made the following observations:

- Relief of ulcer pain after 'Darbid' therapy was "rapid and gratifying."
- ". . . the incidence of side effects was negligible."
- "'Darbid' seems of particular value . . . because its long action simplifies administration."
- ". . . patients receiving 'Darbid' obtained significantly better results than those receiving atropine. . . ."

Dosage in this study was 5 mg. or 10 mg. orally, b.i.d.

Average duration of treatment: 8 months.

1. Cayer, D.; Sohmer, M.F., and Sugg, W.C.: The Effect of Prolonged Continuous Therapy on the Course of Chronic, Recurring Peptic Ulcer; Anticholinergic Therapy with SKF-4740 ('Darbid'), North Carolina M.J. *18*:311 (Aug.) 1957.

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